α-Vinyl Azide—Cysteine Click Coupling Reaction Enabled Bioorthogonal Peptide/Protein Modification

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Supporting Information

Contents

1. General information and materials

1.1 Reagents

Commercial available reagents and solvents were purchased from Energy Chemical, WH Mall or Darui. LookChem and all these reagents were used directly without further purification unless otherwise noted. RP-HPLC solvents were purchased as HPLC grade from Energy Chemical.

1.2 Instruments

NMR spectra were recorded using Bruker AV-300 / AV-400 / AV500 spectrometers. The data are reported as follows: chemical shift in ppm from internal tetramethylsilane on the δ scale, multiplicity (s = singlet, d = doublet, t = triplet, q =quartet, m = multiplet, dd = doublet of doublets, dt = doublet of triplets, td = triplet of doublets), coupling constants (Hz) and integration. High resolution mass spectra were acquired on an agilent 6230 spectrometer and were obtained by peak matching. LC-MS spectra were performed on an Agilent Technologies 1260 InfinityII HPLC system, connected to Agilent 6230B (TOF), equipped with ZORBAX Eclipse Plus C18 column, 4.6×250mm 5-micron, 1.8 μm. Preparative RP-HPLC were performed on Shimadzu LC20AP. Newstyle HPLC systems equipped with LC-20Ap serials

pump, SPD-20A serials UV/VIS detector and CBM-20A System Controller, using the following column: GH0525020C18AB column, 12 nm, 5 μm, 21.2×250 mm. Analytical thin layer chromatography was performed on 0.25 mm extra hard silica gelplates with UV254 fluorescent indicator and/or by exposure to phosphormolybdic acid/cerium (IV) sulfate /ninhydrine followed by brief heating with a heat gun. Liquid chromatography (flash chromatography) was performed on 60Å (40 – 60 μm) mesh silica gel (SiO_2).

All reactions were carried out under nitrogen with anhydrous solvents in oven-dried glassware, unless otherwise noted. All reagents were commercially obtained and, where appropriate, purified prior to use.

1.3 SDS-PAGE analysis

Samples for SDS-PAGE analysis were prepared by mixing protein solution with SDS loading buffer (0.25 M Tris-HCl, 10% SDS, 30% glycerol, and 0.05% bromophenol blue). The samples along with Precision Plus ProteinTM Dual Color Standards were loaded onto hand-cast 10% SDS-PAGE gel (15% acrylamide, 0.375 M Tris (pH 8.8), 0.1% SDS, 0.1% APS and 0.05% TEMED) in SDS running buffer (25 mM Tris, 0.19 M glycine and 0.1% SDS). Fluorescence of protein in the gel was recorded with Gel Doc™ XR+ with image lab™ Software. The gel was stained with elCodeTM Blue Safe Protein Stain.

2. LC-MS and preparative HPLC information

2.1 LC-MS analysis

LC-MS measurements were performed on an Agilent Technologies 1260 Infinity II HPLC system with a G1316A 1260 TCC Column oven, a G1311B 1260 Quat Pump and a G4212B 1260 DAD detector, connected to an Agilent 6230B (TOF), eqiuvuipped with an Agilent ZORBAX SB-C18 column, 4.6×250 mm, 1.8 μm). Water (solvent A) and acetonitrile (solvent B), each containing 0.1% formic acid, were used as the mobile phase. Low-resolution mass spectrometric measurements were acquired using the following parameters: positive electrospray ionization (ESI), temperature of drying gas = 325 °C, flow rate of drying gas=8 L/min, pressure of nebulizer gas = 35 psi, capillary voltage = 3665 V and fragmentor voltage = 175 V. Following LC methods were used:

Method A (Column: Agilent ZORBAX Eclipse Plus C_{18} column, 4.6×250 mm, 1.8 μm, flow rate 0.8 (mL/min)

Time (min)	H_2O (%)	CH_3CN (%)
0	95	5
2	95	5
17	70	30
18	0	0
24	0	0

Method B (Column: Agilent ZORBAX Eclipse Plus C_{18} column, 4.6×250 mm, 1.8 μm, flow rate 0.8 mL/min)

Time (min)	H_2O (%)	CH_3CN (%)

0	99	1
24	99	1

All reported LC-MS yields were determined by integrating TIC spectra. The peak areas for all relevant peptide-containing species on the chromatogram were integrated using Agilent software package. The yields were determined as follows: %yield = Sproduct/Stotal, where Sproduct is the peak area of the product and Stotal is the peak area of combined peptide-containing species (product, starting material and byproduct).

2.2 Preparative HPLC

Preparative HPLC measurements were performed on a Lisure science Ez Plus100D Medium Pressure Preparation System equipped with a SepaFlash column (SW012 Bonded, Spherical C_{18}, 20 - 45μm, 100A) oven, a HP Purifier and UV Preparative Detector. Water (solvent A) and methanol (solvent B) were used as the mobile phase.

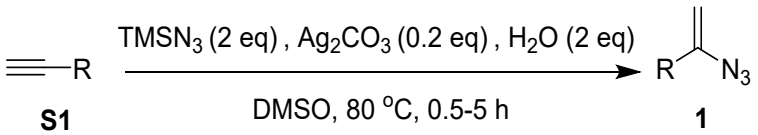
Method C

Time (min)	H_2O (%)	MeOH (%)
0	100	0
10	95	5
45	0	100

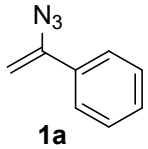
3. Preparation and characterization of substrates

3.1 Preparation of vinyl azides

General procedure

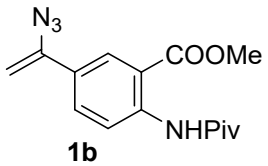


Vinyl azides **1** were prepared from **S1** following Bi's method[1] with slight modification. To a solution of alkyne **S1** (1 equiv), Ag_2CO_3 (0.2 equiv) and H_2O (2 equiv) in DMSO (0.25 M for **S1**) at 80 °C was added $TMSN_3$ (2 equiv). The mixture was stirred under nitrogen atmosphere for 0.5–5 h until **S1** was consumed as indicated by TLC. H_2O was then added. The mixture was extracted with Et_2O three times and the combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. Purification by flash column chromatography on silica gel (PE:EA) gave corresponding vinyl azides.



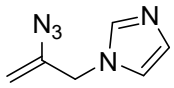
1a

1a: Following the general procedure, the tittle compound was obtained from the reaction of corresponding alkyne in 70% yield as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 – 7.79 (m, 2H), 7.36 – 7.23 (m, 3H), 6.69 (d, J = 4.2 Hz, 1H), 6.33 (d, J = 4.2 Hz, 1H). The data are consistent with those reported in the literature. [2]



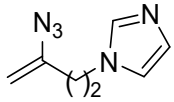
1b

1b: Following the general procedure, the tittle compound was obtained from the reaction of corresponding alkyne in 65% yield as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.37 (s, 1H), 8.78 (d, *J* = 8.9 Hz, 1H), 8.22 (d, *J* = 2.2 Hz, 1H), 7.72 (dd, *J* = 8.9, 2.3 Hz, 1H), 5.44 (d, *J* = 2.6 Hz, 1H), 4.94 (d, *J* = 2.6 Hz, 1H), 3.95 (s, 3H), 1.34 (s, 9H). The data are consistent with those reported in the literature. [2]



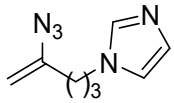
1c

1c: Following the general procedure, the tittle compound was obtained from the reaction of corresponding alkyne in 73% yield as a light yellow oil. ^{1}H NMR (300 MHz, CDCl3) δ 7.49 (s, 1H), 7.08 (s, 1H), 6.92 (s, 1H), 4.86 (m, 2H), 4.42 (s, 2H). ^{13}C NMR (100 MHz, CDCl3) δ 142.7, 137.6, 129.8, 119.2, 100.6, 49.1. HRMS (ESI) m/z Calculated for: $C_6H_8N_3^+$ $[M+H–N_2]^+$122.0713, found 122.0714.



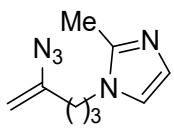
1d

1d: Following the general procedure, the tittle compound was obtained from the reaction of corresponding alkyne in 56% yield as a light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (s, 1H), 7.08 (s, 1H), 6.93 (s, 1H), 4.74 (t, *J* = 1.9 Hz, 2H), 4.10 (t, J = 6.8 Hz, 2H), 2.50 (t, *J* = 6.8 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.7, 137.2, 129.6, 118.7, 100.73, 44.4, 36.2. HRMS (ESI) m/z Calculated for: $C_7H_{10}N_3^+$ $[M+H–N_2]^+$ 136.0869, found 136.0874.



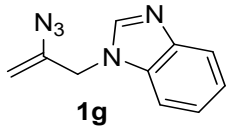
1e

1e: Following the general procedure, the tittle compound was obtained from the reaction of corresponding alkyne in 87% yield as a light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (s, 1H), 7.01 (s, 1H), 6.85 (s, 1H), 4.65 (d, *J* = 5.3 Hz, 2H), 3.89 (t, *J* = 6.7 Hz, 2H), 1.98 (t, *J* = 7.1 Hz, 2H), 1.94 – 1.83 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 145.3, 137.2, 129.7, 118.7, 99.1, 45.7, 30.7, 28.4. HRMS (ESI) m/z Calculated for: $C_8H_{12}N_3^+$ $[M+H–N_2]^+$ 150.1026, found 150.1030.



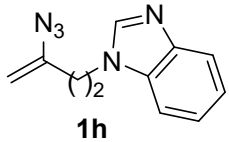
1f

1f: Following the general procedure, the tittle compound was obtained from the reaction of corresponding alkyne in 94% yield as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.95 (s, 1H), 6.85 (s, 1H), 4.76 (s, 1H), 4.74 (s, 1H), 3.88 (t, *J* = 7.1 Hz, 2H), 2.41 (s, 3H), 2.11 (t, *J* = 7.3 Hz, 2H), 1.98 – 1.88 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.5, 144.4, 127.3, 119.0, 98.9, 44.9, 30.8, 28.1, 13.1.HRMS (ESI) m/z Calculated for: $C_9H_{14}N_3^+$ $[M+H–N_2]^+$ 164.1182, found 164.1187.



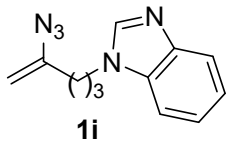
1g

1g: Following the general procedure, the tittle compound was obtained from the reaction of corresponding alkyne in 82% yield as a yellow oil. ^{1}H NMR (400 MHz, CDCl3) δ 7.90 (s, 1H), 7.86 – 7.77 (m, 1H), 7.42 – 7.36 (m, 1H), 7.35 – 7.27 (m, 2H), 5.03 – 4.80 (m, 2H), 4.65 (s, 2H). ^{13}C NMR (100 MHz, CDCl3) δ 143.9, 143.3, 141.8, 133.7, 123.3, 122.5, 120.6, 109.8, 100.5, 47.2. HRMS (ESI) m/z Calculated for: $C_{10}H_{10}N_3^+$ $[M+H–N_2]^+$ 172.0869, found 172.0873.



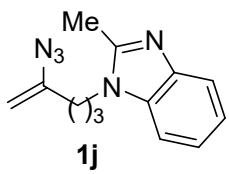
1h

1h: Following the general procedure, the tittle compound was obtained from the reaction of corresponding alkyne in 85% yield as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (s, 1H), 7.90 – 7.82 (m, 1H), 7.48 – 7.42 (m, 1H), 7.40 – 7.32 (m, 2H), 4.77 – 4.49 (m, 2H), 4.36 (t, *J* = 6.8 Hz, 2H), 2.62 (t, *J* = 6.8 Hz, 2H). ^{13}C NMR (75 MHz, CDCl3) δ 143.9, 143.0, 142.7, 123.0, 122.2, 120.6, 109.4, 100.9, 42.6, 34.8. HRMS (ESI) m/z Calculated for: $C_{11}H_{12}N_3^+$ $[M+H–N_2]^+$ 186.1026, found 186.1024.



1i

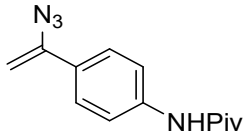
1i: Following the general procedure, the tittle compound was obtained from the reaction of corresponding alkyne in 87% yield as a light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (s, 1H), 7.82 (dd, *J* = 6.5, 2.2 Hz, 1H), 7.43 – 7.35 (m, 1H), 7.34 – 7.26 (m, 2H), 4.75 – 4.66 (m, 2H), 4.19 (t, *J* = 6.7 Hz, 2H), 2.15 – 2.04 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.3, 144.0, 143.0, 133.7, 123.0, 122.2, 120.6, 109.6, 99.1, 43.9, 30.9, 27.1. HRMS (ESI) m/z Calculated for: $C_{12}H_{14}N_3^+$ $[M+H–N_2]^+$ 228.1244, found 228.1245.



1j

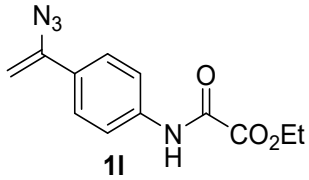
1j: Following the general procedure, the tittle compound was obtained from the reaction of corresponding alkyne in 91% yield as a light yellow oil. ^{1}H NMR (400

MHz, $CDCl_3$) δ 7.75 – 7.61 (m, 1H), 7.32 – 7.26 (m, 1H), 7.25 – 7.18 (m, 2H), 4.74 (s, 1H), 4.71 (s, 1H), 4.11 (t, *J* = 7.4 Hz, 2H), 2.60 (s, 3H), 2.13 (t, *J* = 7.4 Hz, 2H), 2.06 – 1.90 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.3, 145.5, 142.7, 135.1, 122.1, 121.9, 119.2, 109.0, 98.8, 42.9, 31.2, 27.2, 14.0. HRMS (ESI) m/z Calculated for: $C_{13}H_{16}N_3^+$ $[M+H–N_2]^+$ 214.1339, found 214.1339.



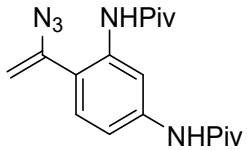
1k

1k: Following the general procedure, the tittle compound was obtained from the reaction of corresponding alkyne in 63% yield as a white solid. ^1H NMR (400 MHz, $CDCl_3$) δ 7.51 (q, *J* = 8.6 Hz, 4H), 5.37 (s, 1H), 4.89 (s, 1H), 1.30 (s, 9H). The data are consistent with those reported in the literature. [2]



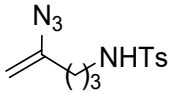
1l

1l: Following the general procedure, the tittle compound was obtained from the reaction of corresponding alkyne in 80% yield as a white solid. ^1H NMR (400 MHz, $CDCl_3$) δ 8.95 (s, 1H), 7.69 – 7.50 (m, 4H), 5.42 (d, *J* = 2.4 Hz, 1H), 4.94 (d, *J* = 2.4 Hz, 1H), 4.41 (q, *J* = 7.1 Hz, 2H), 1.42 (t, *J* = 7.1 Hz, 3H). The data are consistent with those reported in the literature. [2]



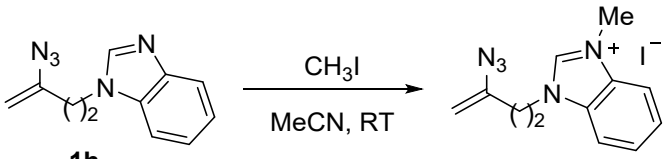
1m

1m: Following the general procedure, the tittle compound was obtained from the reaction of corresponding alkyne in 49% yield as a yellow solid. ^1H NMR (400 MHz, $CDCl_3$) δ 8.36 (s, 1H), 8.26 (d, *J* = 2.1 Hz, 1H), 7.85 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.47 – 7.36 (m, 2H), 3.51 (s, 1H), 1.33 (s, 9H), 1.28 (s, 9H). The data are consistent with those reported in the literature. [2]



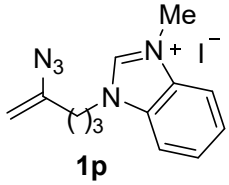
1n

1n: Following the general procedure, the tittle compound was obtained from the reaction of corresponding alkyne in 77% yield as a white solid. ^1H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, J = 8.1 Hz, 2H), 7.29 (d, *J* = 8.1 Hz, 2H), 4.61–4.58 (m, 2H), 2.91 (t, J = 6.9 Hz, 2H), 2.41 (s, 3H), 2.03 (t, *J* = 7.3 Hz, 2H), 1.65–1.58 (m, 2H). The data are consistent with those reported in the literature. [2]



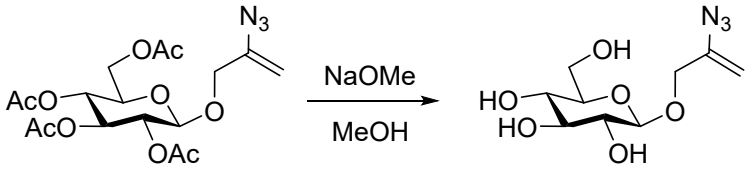
1h **1o**

Typical procedure for iodomethane salt of vinyl azides 1o: The interior of a sealing tube was provided with a stir bar. **1h** (2.1 mmol), iodomethane (10.5 mmol) and acetonitrile (6 mL) were added. The mixture was stirred at room temperature until **1h** was consumed (2 days, indicated by TLC). The suspension was evaporated under reduced pressure to 1 mL and filtered. The residual solid was washed with ethyl acetate (15 mL×3) and dried in vacuo to afford a white solid **1o** (660 mg, 93%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.98 (s, 1H), 7.97 – 7.72 (m, 2H), 7.72 – 7.58 (m, 2H), 5.02 (d, *J* = 1.1 Hz, 1H), 4.73 (t, *J* = 6.8 Hz, 2H), 4.67 (d, *J* = 2.1 Hz, 1H), 4.28 (s, 3H), 2.91 (t, *J* = 6.8 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 142.5, 141.5, 131.8, 131.3, 127.5, 127.4, 113.1, 112.8, 102.3, 45.0, 34.4, 34.1. HRMS (ESI) m/z Calculated for: $C_{12}H_{14}N_3^+$ $[M+H–N_2]^+$ 228.1244, found 228.1258.



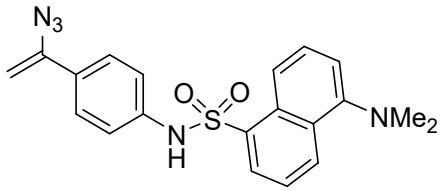
1p

1p: According to the typical procedure described for **1o**, **1i** (500 mg, 2.2 mmol) was converted into **1p** (792 mg, 91% yield) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.94 (s, 1H), 7.81 – 7.71 (m, 2H), 7.70 – 7.63 (m, 2H), 4.86 (s, 1H), 4.67 (s, 1H), 4.64 (t, *J* = 7.1 Hz, 2H), 4.28 (s, 3H), 2.27 (s, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 144.7, 142.2, 132.0, 131.1, 127.5, 113.2, 113.1, 99.8, 46.7, 34.4, 30.8, 26.6. HRMS (ESI) m/z Calculated for: $C_{13}H_{16}N_3^+$ $[M+H–N_2]^+$ 214.1339, found 214.1344.



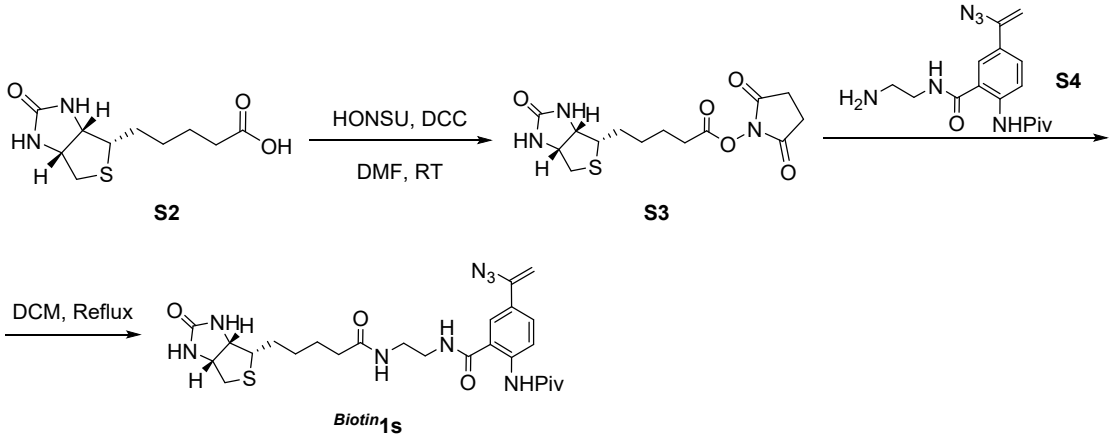
1q' **1q**

1q: According to the general procedure described for the preparation of **1**, **1q'** was obtained as a white solid. A flask was provided with a stir bar, **1q'** (400 mg, 0.93 mmol) and 5 mL of MeOH (dry) and cooled to 0 °C. Then NaOMe (3M in MeOH, 0.19 mmol, 62 μL) was added. The mixture was allowed to warm to rt and stirred for 3 h under argon, neutralized with Spelite LXP-13 (H^+) resin, filtered and evaporated to dryness, providing 90% yield (0.84 mmol, 359 mg) of **1q**. ^{1}H NMR (400 MHz, MeOD) δ 4.92 (s, 1H), 4.84 – 4.79 (m, 2H), 4.20 (d, 1H), 4.07 (d, *J* = 12.8 Hz, 1H), 3.84 (dd, *J* = 11.8, 1.8 Hz, 2H), 3.74 – 3.66 (m, 2H), 3.62 (t, *J* = 9.4 Hz, 1H), 3.58 – 3.50 (m, 1H). ^{13}C NMR (100 MHz, MeOD) δ 142.5, 100.8, 99.3, 73.7, 71.1, 70.6, 67.1, 66.2, 61.5. HRMS (ESI) m/z Calculated for: $C_9H_{15}N_3NaO_6^+$ $[M+Na]^+$ 284.0853, found 284.0848.



1r

1r: Following the general procedure described for the preparation of **1**, the tittle compound was obtained from the reaction of corresponding alkyne in 65% yield as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.51 (d, *J* = 8.5 Hz, 1H), 8.39 (d, *J* = 8.7 Hz, 1H), 8.24 (d, *J* = 7.3 Hz, 1H), 7.55 (dd, *J* = 8.9, 7.3 Hz, 2H), 7.48 – 7.41 (m, 1H), 7.29 (d, *J* = 8.7 Hz, 2H), 7.16 (d, *J* = 7.6 Hz, 1H), 6.96 (d, *J* = 8.7 Hz, 2H), 5.26 (d, *J* = 2.5 Hz, 1H), 4.83 (d, J = 2.5 Hz, 1H), 2.86 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.1, 144.1, 137.4, 134.0, 131.1, 130.7, 130.4, 129.9, 129.6, 128.8, 126.4, 123.1, 120.2, 118.4, 115.4, 97.4, 45.4. HRMS (ESI) m/z Calculated for: $C_{20}H_{19}N_5NaO_2S^+$ $[M+Na]^+$ 416.1152, found 416.1156.



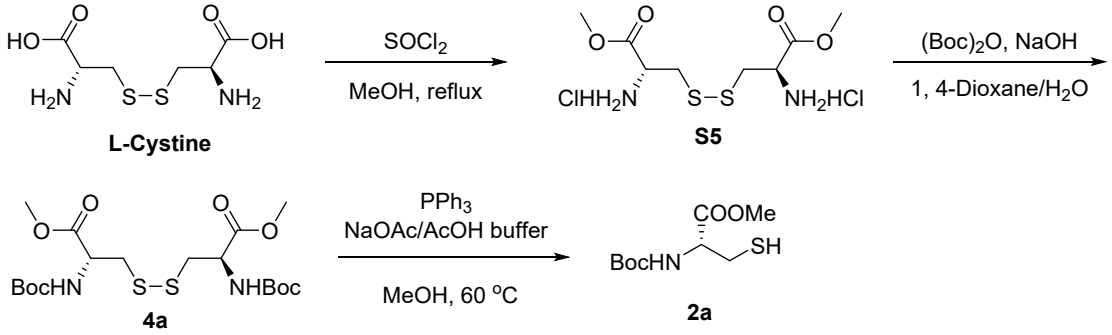
S2 **S3** **S4** **^{Biotin}1s**

(+)-Biotin **S2** (2 g, 8.2 mmol) and N-hydroxysuccinimide (942 mg, 1 equiv) were dissolved in dry DMF (40 mL). N, N'-Dicyclohexylcarbodiimide (2.2 g, 1.3 equiv) was added to the solution and the mixture was stirred overnight at room temperature. The reaction mixture was filtered through celite. DMF was evaporated and the resulting residue was left under vacuum for a few hours and then washed with ether and dried under vacuum to yield **S3** (2.5 g, 89%).

***Biotin*1s:** Biotin-NHS **S3** (400 mg, 1.2 mmol) and N-(2-aminoethyl)-5-(1-azidovinyl)-2-pivalamido-benzamide **S4** (1.24 g, 3 equiv) were dissolved in dry DCM (10 mL) and refluxed until Biotin-NHS was consumed. The reaction mixture was quenched with water (50 mL) and extracted with DCM (40 mL×2). Organic layer was washed with brine (50 mL), dried over anhydrous Na_2SO_4 and concentrated in vacuo. Purification by flash column chromatography on silica gel (MeOH:DCM = 1:10) gave 小 in 75% yield as a yellow solid. NMR showed the presence of at least two isomers. Major isomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ11.54 (s, 1H) 8.52 (d, *J* = 8.8 Hz, 1H), 8.41 (s, 1H), 7.82 (s, 1H), 7.54 (d, *J* = 9.0 Hz, 1H), 7.38 (s, 1H), 6.74 (s, 1H), 6.12 (s, 1H), 5.50 (s, 1H), 4.85 (s, 1H), 4.42 – 4.26 (m, 1H), 4.14 – 4.02 (m, 1H), 3.49 – 3.27 (m, 4H), 2.98 – 2.86 (m, 1H), 2.84 – 2.67 (m, 1H), 2.65 – 2.48 (m, 1H), 2.17 – 2.03

(m, 2H), 1.61 – 1.41 (m, 6H), 1.24 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.0, 175.0, 169.3, 164.4, 143.6, 140.5, 129.1, 128.3, 124.7, 120.8, 120.0, 97.6, 61.7, 60.2, 55.7, 41.1, 40.2, 39.0, 35.8, 32.5, 28.2, 27.6, 26.1, 25.5. Minor isomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ11.54 (s, 1H), 8.52 (d, J = 8.8 Hz, 1H), 8.41 (s, 1H), 7.82 (s, 1H), 7.54 (d, J = 9.0 Hz, 1H), 7.38 (s, 1H), 7.02 (s, 1H), 6.55 (s, 1H), 6.22 (s, 1H), 6.12 (s, 1H), 4.42 – 4.26 (m, 1H), 4.22 – 4.15 (m, 1H), 3.87 (t, J = 10.2 Hz, 1H), 3.72 – 3.47 (m, 3H), 3.09 – 2.99 (m, 1H), 2.84 – 2.67 (m, 1H), 2.65 – 2.48 (m, 1H), 2.34 – 2.20 (m, 2H),1.92 – 1.81 (m, 2H), 1.75 – 1.65 (m, 4H), 1.24 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.0, 175.0, 164.4, 154.0, 143.6, 140.5, 129.1, 128.3, 124.7, 120.8, 120.0, 97.6, 61.7, 60.3, 55.6, 40.5, 40.2, 39.0, 35.8, 30.9, 28.0, 27.6, 26.1, 24.8. HRMS (ESI) m/z Calculated for: $C_{26}H_{36}N_8NaO_4S^+$ $[M+Na]^+$ 579.2472, found 579.2468.

3.2 Preparation of peptides



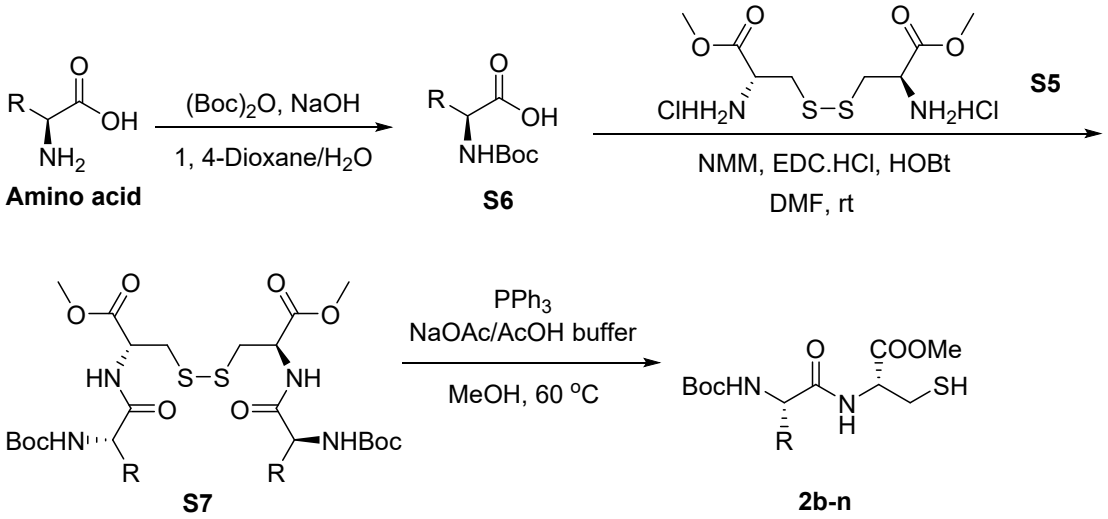
S5: L-Cystine (5 g, 20 mmol) was added to a 250 mL round-bottom flask followed by 120 mL of methanol. The flask was cooled in an ice-bath and thionyl chloride (7.5 mL, 1 mol, 5 equiv) was added. The reaction mixture was then heated to reflux for 6 h. The solvent was removed in vacuo. The crude product was washed with EA (50 mL×2) and dried in vacuo to afford **S5** as a white solid (7.1 g, 99%), which was used directly in the next reaction.

4a: S5 (1 g, 2.9 mmol, 1.0 equiv) was added into a 100 mL round-bottom flask, followed by NaOH (351 mg, 8.8 mmol), H_2O (8 mL) and 1, 4-Dioxane (8 mL). The flask was cooled in an ice-bath, then Boc_2O (0.8 mL, 1.2 mmol) was added dropwise. The reaction mixture was allowed to warm to room temperature and was stirred for 12 h. The suspension was evaporated under reduced pressure. The resulting residue was dispersed in H_2O (20 mL) and extracted with EA (50 mL×2). Organic layer was dried over anhydrous Na_2SO_4. Concentration in vacuo afforded **4a** in 99% yield as a transparent viscous solid.

2a: To a solution of **4a** (1.6 g, 3.4 mmol, 1.0 equiv) in MeOH (14 mL) and NaOAc/ AcOH (pH 4.9) buffer (7 mL) was added triphenylphosphine (1.07 g, 1.2 equiv). The reaction mixture was stirred at 60 °C for 16 h. Then it was cooled to rt, diluted with DCM (50 mL) and washed with water (20 mL×2) . The organic layer was dried over anhydrous Na_2SO_4, concentrated and purified by flash column chromatography on silica gel (EtOAc/PE) to provide **2a** (1.5 g, 5.0 mmol, 93% yield) as a transparent viscous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.39 (d, J = 7.4 Hz, 1H), 4.62 – 4.48 (m,

1H), 3.74 (s, 3H), 3.14 (d, J = 4.9 Hz, 2H), 1.43 (s, 9H). The data are consistent with those reported in the literature.[3]

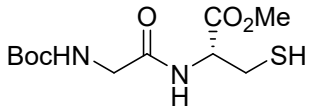
General procedure for 2b–n



S6: To a 100 mL round-bottom flask, was added **Amino acid** (5 mmol, 1.0 equiv), NaOH (3.0 equiv), H_2O (8 mL) and 1, 4-Dioxane (10 mL). The flask was cooled in an ice-bath, then Boc_2O (0.8 mL, 1.2 mmol) was added dropwise. The reaction mixture was allowed to warm to room temperature and was stirred for 12 h. The suspension was evaporated under reduced pressure. The resulting residue was dispersed in H_2O (20 mL) and adjusted to pH 2-3 using 1N HCl. Then it was extracted with EA (50 mL×2). Organic layer was dried over anhydrous Na_2SO_4. Concentration in vacuo afforded **S6** in almost quantitative yield, which was used directly in the next reaction.

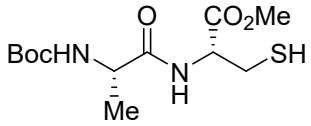
S7: To a 25 mL round-bottom flask, was added **S6** (1.1 mmol, 2.2 equiv) and DMF (6 mL). The flask was cooled in an ice-bath, then 1-hydroxybenzotriazole hydrate (HOBt) (1.1 mmol, 2.2 equiv) was added. The mixture was stirred at 0 °C until all HOBt dissolved. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl) (1.1 mmol, 2.2 equiv) was added. The mixture was stirred at 0 °C for 5 min before adding **S5** (0.50 mmol, 1.0 equiv) and N-methylmorpholine (NMM) (2.2 mmol, 2.2 equiv). The resulting reaction mixture was stirred at 0 °C for 90 min plus room temperature 16 h. EtOAc (20 mL) was added and the mixture was washed with water (25 mL×4). The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo. Flash chromatography on silica gel (EtOAc/PE) afforded **S7** in 30-80%.

2b-n: To a solution of **S7** (1.0 equiv) in MeOH (18 mL) and NaOAc/ AcOH (pH 4.9) buffer (9 mL) was added triphenylphosphine (1.2 equiv). The reaction mixture was stirred at 60 °C for 2 h. Then it was cooled to room temperature and extracted with EtOAc (20 mL×4) The organic layer was dried over anhydrous Na_2SO_4, concentrated and purified by flash column chromatography on silica gel (EtOAc/ PE) to provide **2b-n**.[4]



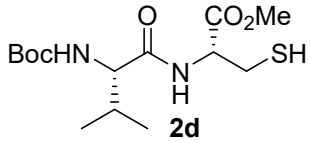
2b

2b: Following the general procedure, the tittle compound was obtained from the reaction of corresponding **S7** in 90% yield as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.13 (s, 1H), 5.37 (t, *J* = 5.6 Hz, 1H), 4.94 – 4.72 (m, 1H), 3.88 – 3.78 (m, 2H), 3.76 (s, 3H), 3.04 – 2.95 (m, 2H), 1.43 (s, 9H). The data are consistent with those reported in the literature.[4]



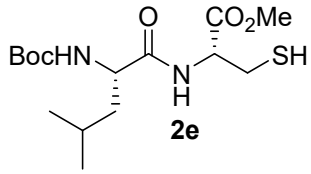
2c

2c: Following the general procedure, the tittle compound was obtained from the reaction of corresponding **S7** in 96% yield as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.00 (s, 1H), 5.02 (s, 1H), 4.87 – 4.80 (m, 1H), 4.24 – 4.13 (m, 1H), 3.78 (s, 3H), 3.06 – 2.91 (m, 2H), 1.44 (s, 9H), 1.37 (d, *J* = 7.1 Hz, 3H). The data are consistent with those reported in the literature.[4]



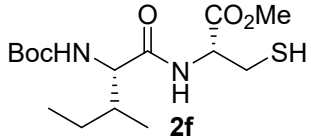
2d

2d: Following the general procedure, the tittle compound was obtained from the reaction of corresponding **S7** in 93% yield as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.83 (d, *J* = 6.6 Hz, 1H), 5.07 (d, *J* = 7.6 Hz, 1H), 4.94 – 4.74 (m, 1H), 4.03 – 3.91 (m, 1H), 3.78 (s, 3H), 3.14 – 2.90 (m, 2H), 2.25 – 1.98 (m, 1H), 1.44 (s, 9H), 0.98 (d, *J* = 6.8 Hz, 3H), 0.94 (d, *J* = 6.8 Hz, 3H). The data are consistent with those reported in the literature.[4]



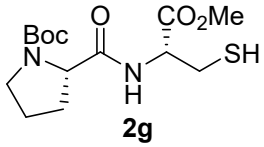
2e

2e: Following the general procedure, the tittle compound was obtained from the reaction of corresponding **S7** in 80% yield as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.99 – 6.88 (m, 1H), 4.94 – 4.87 (m, 1H), 4.87 – 4.80 (m, 1H), 4.17 – 4.04 (m, 1H), 3.78 (s, 3H), 3.09 – 2.86 (m, 2H), 1.74 – 1.62 (m, 2H), 1.56 – 1.46 (m, 2H), 1.44 (s, 9H), 0.97 – 0.78 (m, 6H). The data are consistent with those reported in the literature.[4]



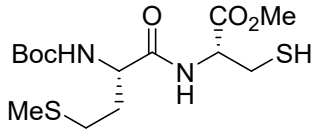
2f

2f: Following the general procedure, the tittle compound was obtained from the reaction of corresponding **S7** in 73% yield as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.75 (d, *J* = 6.7 Hz, 1H), 5.01 (d, *J* = 7.3 Hz, 1H), 4.93 – 4.78 (m, 1H), 4.07 – 3.93 (m, 1H), 3.79 (s, 3H), 3.13 – 2.84 (m, 2H), 2.03 – 1.68 (m, 2H), 1.52 – 1.47 (m, 1H), 1.45 (s, 9H), 1.21 – 1.11 (m, 1H), 1.05 – 0.80 (m, 6H). The data are consistent with those reported in the literature.[4]



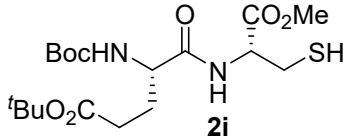
2g

2g: Following the general procedure, the tittle compound was obtained from the reaction of corresponding **S7** in 72% yield as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 – 7.42 (m, 1H), 4.87 – 4.74 (m, 1H), 4.34 – 4.17 (m, 1H), 3.76 (s, 3H), 3.52 – 3.25 (m, 2H), 3.06 – 2.86 (m, 2H), 2.31 – 2.08 (m, 1H), 2.03 – 1.80 (m, 3H), 1.45 (s, 9H), 1.35 – 1.16 (m, 1H). The data are consistent with those reported in the literature.[4]



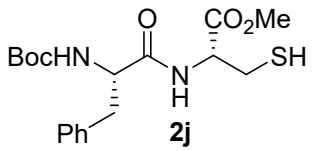
2h

2h: Following the general procedure, the tittle compound was obtained from the reaction of corresponding **S7** in 82% yield as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.02 (d, *J* = 4.7 Hz, 1H), 5.18 (d, *J* = 5.9 Hz, 1H), 4.92 – 4.61 (m, 1H), 4.34 – 4.05 (m, 1H), 3.73 (s, 3H), 2.96 (d, *J* = 3.8 Hz, 1H), 2.93 (d, *J* = 3.8 Hz, 1H), 2.54 (t, *J* = 7.1 Hz, 2H), 2.09 – 1.98 (m, 4H), 1.96 – 1.85 (m, 1H), 1.39 (s, 9H). The data are consistent with those reported in the literature.[4]



2i

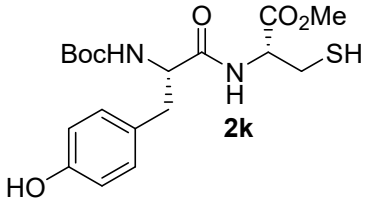
2i: Following the general procedure, the tittle compound was obtained from the reaction of corresponding **S7** in 91% yield as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.86 (d, *J* = 6.4 Hz, 1H), 5.23 (d, *J* = 7.2 Hz, 1H), 4.91 – 4.79 (m, 1H), δ 4.33 – 4.08 (m, 1H), 3.76 (s, 3H), 3.08 – 2.91 (m, 2H), 2.33 (t, *J* = 7.2 Hz, 2H), 2.25 – 2.13 (m, 1H), 1.89 – 1.76 (m, 1H), 1.57 (t, *J* = 8.9 Hz, 1H), 1.44 (s, 9H), 1.42 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.96, 171.39, 170.58, 155.83, 82.23, 79.91, 67.92, 53.87, 52.73, 32.30, 28.31, 27.97, 26.71, 25.58. HRMS (ESI) m/z Calculated for: $C_{18}H_{32}N_2NaO_7S^+$ $[M+Na]^+$ 443.1822, found 443.1827.



2j

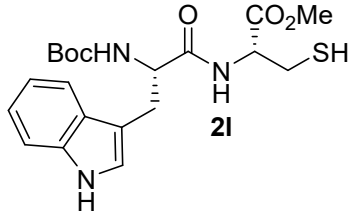
2j: Following the general procedure, the tittle compound was obtained from the

reaction of corresponding **S7** in 89% yield as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37 – 7.30 (m, 2H), 7.29 – 7.19 (m, 3H), 6.85 (s, 1H), 5.03 (s, 1H), 4.86 – 4.77 (m, 1H), 4.42 (d, 1H), 3.77 (s, 3H), 3.20 – 3.12 (m, 1H), 3.12 – 3.06 (m, 1H), 3.06 – 2.89 (m, 2H), 1.44 (s, 9H). The data are consistent with those reported in the literature.[4]



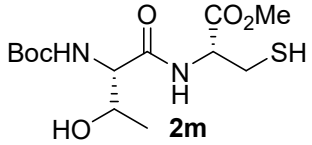
2k

2k: Following the general procedure, the tittle compound was obtained from the reaction of corresponding **S7** in 89% yield as a yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.06 (d, J = 7.9 Hz, 2H), 6.83 (d, J = 6.4 Hz, 1H), 6.78 (d, J = 8.0 Hz, 2H), 5.06 (s, 1H), 4.80 (s, 1H), 4.43 – 4.29 (m, 1H), 3.77 (s, 3H), 3.13 – 3.04 (m, 1H), 3.03 – 2.91 (m, 3H), 1.46 (s, 9H). The data are consistent with those reported in the literature.[4]



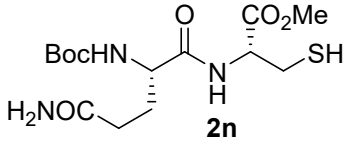
2l

2l: Following the general procedure, the tittle compound was obtained from the reaction of corresponding **S7** in 69% yield as a white solid. ^{1}H NMR (500 MHz, CDCl3) δ 7.61 (d, J = 7.9 Hz, 1H), 7.40 – 7.29 (m, 1H), 7.23 – 7.12 (m, 1H), 7.13 – 7.01 (m, 2H), 5.43 – 5.29 (m, 1H), 4.83 – 4.45 (m, 2H), 3.70 – 3.59 (m, 3H), 3.37 – 3.17 (m, 2H), 2.95 – 2.76 (m, 1H), 2.05 (s, 1H), 1.41 (s, 9H). The data are consistent with those reported in the literature.[4]



2m

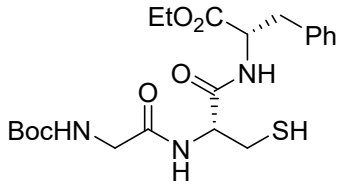
2m: Following the general procedure, the tittle compound was obtained from the reaction of corresponding **S7** in 80% yield as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (d, J = 7.3 Hz, 1H), 5.62 (d, J = 7.6 Hz, 1H), 4.89 – 4.75 (m, 1H), 4.39 – 4.25 (m, 1H), 4.14 (d, J = 6.6 Hz, 1H), 3.76 (s, 3H), 3.55 (s, 1H), 3.05 – 2.86 (m, 2H), 1.51 (t, J = 9.0 Hz, 1H), 1.43 (s, 9H), 1.18 (d, J = 6.3 Hz, 3H). The data are consistent with those reported in the literature.[4]



2n

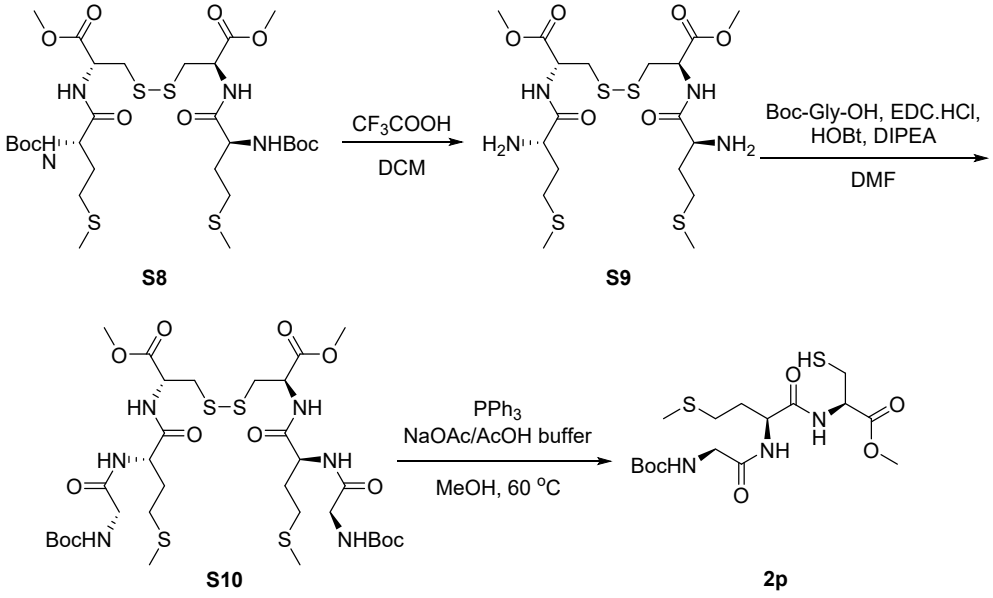
2n: Following the general procedure, the tittle compound was obtained from the reaction of corresponding **S7** in 90% yield as a white solid. ^{1}H NMR (400 MHz,

$CDCl_3$) δ 8.00 (d, *J* = 5.2 Hz, 1H), 7.00 (s, 1H), 6.43 (s, 1H), 5.72 (s, 1H), 4.94 – 4.73 (m, 1H), 4.30 (s, 1H), 3.76 (s, 3H), 3.11 – 2.81 (m, 2H), 2.62 – 2.34 (m, 2H), 2.15 – 1.92 (m, 2H), 1.79 (t, *J* = 8.4 Hz, 1H), 1.42 (s, 9H). The data are consistent with those reported in the literature.[4]



2o

2o: The experimental steps refer to Halcomb's method.[5] The product **2o** was afforded as a white solid (200 mg,88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 – 7.08 (m, 7H), 5.41 (s, 1H), 4.86 – 4.76 (m, 1H), 4.72 – 4.61 (m, 1H), 4.14 (q, *J* = 7.1 Hz, 2H), 3.86 – 3.67 (m, 2H), 3.15 (dd, *J* = 13.9, 5.7 Hz, 1H), 3.04 (dd, *J* = 13.9, 7.3 Hz, 1H), 3.00 – 2.89 (m, 1H), 2.73 – 2.61 (m, 1H), 1.62 – 1.49 (m, 1H), 1.44 (s, 9H), 1.21 (t, *J* = 7.1 Hz, 3H). The data are consistent with those reported in the literature.[5]

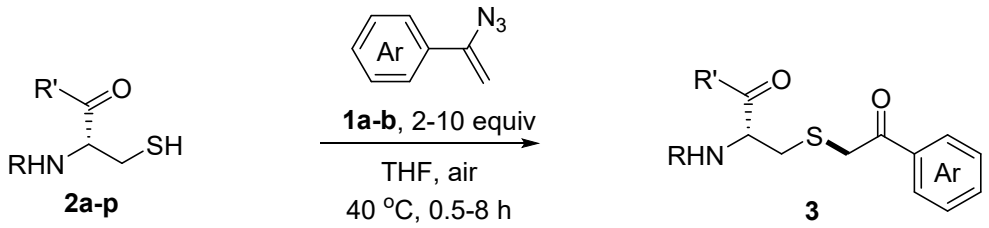


S10: To a 100 mL round-bottom flask, was added **S8** (400 mg, 0.55 mmol), CH_2Cl_2 (5 mL), trifluoroacetic acid (5 mL). The reaction mixture was stirred for 1 h, the TFA was removed under reduced pressure and the resulting brown residue was dried under vacuum for 1 h to give crude **S9**. It was then dissolved in DMF (10 mL) and treated with HOBt (185 mg, 1.37 mmol), EDC·HCl (264 mg, 1.37 mmol), Boc-Gly-OH (240 mg, 1.37 mmol) and N, N-Diisopropylethylamine (DIPEA) (0.21 mL, 1.37 mmol). The resulting reaction mixture was stirred at rt for 36 h. The mixture was added EtOAc (50 mL) and washed with NH_4Cl aq. (50 mL), $NaHCO_3$ aq. (50 mL) and NaCl aq. (50 mL). The organic layer was dried over anhydrous Na_2SO_4, concentrated and purified by flash column chromatography on silica gel (MeOH/DCM) to provide **S10** in 59% as a white solid.

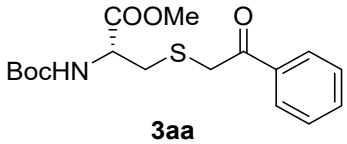
2p: To a solution of **S10** (1.0 equiv) in MeOH (18 mL) and NaOAc/AcOH (pH 4.9)

buffer (9 mL) was added triphenylphosphine (1.2 equiv). The reaction mixture was stirred at 60 °C for 2 h. Then it was cooled to room temperature and extracted with EtOAc (20 mL×4). The organic layer was dried over anhydrous Na_2SO_4, concentrated and purified by flash column chromatography on silica gel (MeOH/DCM) to provide **2p** as a white solid (72%) after it was dried overnight under high vacuum. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (s, 1H), 7.45 (s, 1H), 5.67 (s, 1H), 4.96 – 4.71 (m, 2H), 3.96 – 3.82 (m, 1H), 3.82 – 3.76 (m, 1H), 3.74 (s, 3H), 3.11 (s, 2H), 2.57 (t, *J* = 6.9 Hz, 2H), 2.14 (s, 1H), 2.09 (s, 3H), 2.04 – 1.92 (m, 1H), 1.43 (s, 9H). The data are consistent with those reported in the literature.[4]

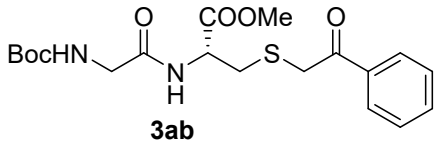
4. Modification of cysteine-containing peptides/proteins by vinyl azides



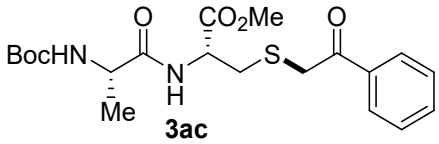
General procedure I: To a 10 mL round-bottom flask, were added peptide **2** (0.2 mmol), THF (3 mL) and vinyl azide **1** (2 mmol, 2-10 eqiuv). The reaction mixture was stirred at 40 °C in the air atmosphere until the peptide was consumed (monitored by TLC). Then it was cooled to room temperature, concentrated and purified by flash column chromatography on silica gel (EA/PE) to provide product **3**.



3aa: According to **General procedure I**, **3aa** was afforded as a white soild (77% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.00 – 7.90 (m, 2H), 7.63 – 7.54 (m, 1H), 7.51 – 7.39 (m, 2H), 5.42 (d, *J* = 7.5 Hz, 1H), 4.56 (dd, *J* = 12.4, 5.6 Hz, 1H), 3.88 (s, 2H), 3.71 (s, 3H), 3.06 (dd, *J* = 14.0, 4.8 Hz, 1H), 2.96 (dd, *J* = 14.0, 6.1 Hz, 1H), 1.43 (s, 9H). The data are consistent with those reported in the literature.[6]

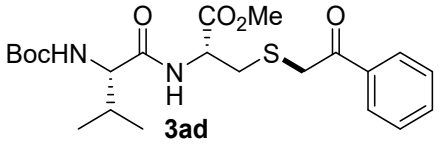


3ab: According to t **General procedure I**, **3ab** was afforded as a white oil (61% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 – 7.84 (m, 2H), 7.55 – 7.48 (m, 1H), 7.43 – 7.36 (m, 2H), 5.31 (s, 1H), 4.87 – 4.69 (m, 1H), 3.95 – 3.69 (m, 4H), 3.64 (s, 3H), 3.08 – 2.85 (m, 2H), 1.37 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.6, 170.9, 169.8, 156.0, 135.1, 133.7, 132.1, 132.0, 128.8, 128.7, 80.1, 52.8, 52.0, 44.2, 37.8, 34.0, 28.3. HRMS (ESI) m/z Calculated for: $C_{19}H_{27}N_2O_6S^+$ $[M+H]^+$ 411.1584, found 411.1585.



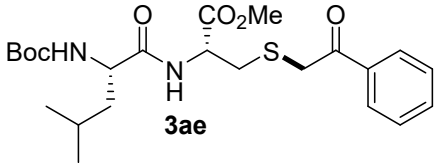
3ac

3ac: According to **General procedure I**, **3ac** was afforded as a white oil (30% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 – 7.86 (m, 2H), 7.64 – 7.51 (m, 1H), 7.46 (t, *J* = 7.7 Hz, 2H), 7.21 (d, *J* = 6.5 Hz, 1H), 5.12 (s, 1H), 4.87 – 4.74 (m, 1H), 4.24 (s, 1H), 3.93 (d, *J* = 14.9 Hz, 1H), 3.87 (d, *J* = 14.8 Hz, 1H), 3.70 (s, 3H), 3.12 – 2.93 (m, 2H), 1.43 (s, 9H), 1.38 (d, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.7, 172.8, 170.8, 155.4, 135.1, 133.7, 128.8, 128.7, 80.1, 52.7, 52.0, 50.1, 37.8, 34.3, 28.3, 18.4. HRMS (ESI) m/z Calculated for: $C_{20}H_{28}N_2NaO_6S^+$ $[M+Na]^+$ 447.1560, found 447.1552.



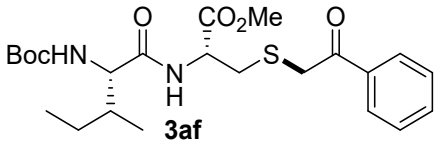
3ad

3ad: According to **General procedure I I**, **3ad** was afforded as a yellow viscous oil (64% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, *J* = 7.6 Hz, 2H), 7.58 (t, *J* = 7.3 Hz, 1H), 7.46 (t, *J* = 7.7 Hz, 2H), 7.16 (d, *J* = 7.5 Hz, 1H), 5.15 (d, *J* = 8.5 Hz, 1H), 4.83 (dd, *J* = 12.8, 5.4 Hz, 1H), 4.12 – 4.01 (m, 1H), 3.94 (d, *J* = 14.9 Hz, 1H), 3.88 (d, *J* = 14.9 Hz, 1H), 3.70 (s, 3H), 3.03 (d, *J* = 5.3 Hz, 2H), 2.25 – 2.11 (m, 1H), 1.42 (s, 9H), 0.99 (d, *J* = 6.8 Hz, 3H), 0.93 (d, *J* = 6.8 Hz, 3H).^{13}C NMR (100 MHz, $CDCl_3$) δ 194.7, 171.7, 170.8, 155.8, 135.0, 133.8, 128.8, 128.78, 79.8, 59.7, 52.7, 52.0, 37.7, 34.1, 31.1, 28.3, 19.2, 17.7. HRMS (ESI) m/z Calculated for: $C_{22}H_{33}N_2O_6S^+$ $[M+H]^+$ 453.2054, found 453.2069.



3ae

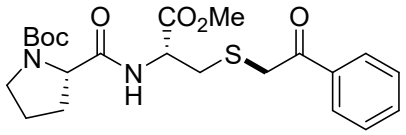
3ae: According to **General procedure I**, **3ae** was afforded as a yellow viscous oil (44% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (d, *J* = 7.7 Hz, 2H), 7.57 (t, *J* = 7.4 Hz, 1H), 7.45 (t, *J* = 7.4 Hz, 2H), 7.25 (s, 1H), 5.01 (s, 1H), 4.82 (dd, *J* = 12.5, 5.6 Hz, 1H), 4.19 (s, 1H), 3.94 (d, *J* = 14.9 Hz, 1H), 3.87 (d, *J* = 14.9 Hz, 1H), 3.69 (s, 3H), 3.07 – 2.97 (m, 2H), 1.76 – 1.61 (m, 2H), 1.52 – 1.45 (m, 1H), 1.41 (s, 9H), 0.94 (s, 3H), 0.92 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.7, 172.7, 170.8, 155.6, 135.1, 133.7, 128.8, 128.75, 80.0, 53.1, 52.7, 52.0, 41.4, 37.9, 34.3, 28.3, 24.72, 23.0, 22.0. HRMS (ESI) m/z Calculated for: $C_{23}H_{34}N_2NaO_6S^+$ $[M+Na]^+$ 489.2030, found 489.2034.



3af

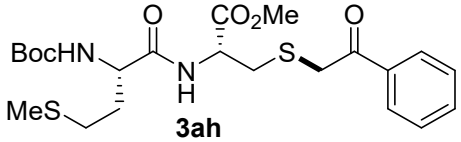
3af: According to **General procedure I**, **3af** was afforded as a yellow oil (52% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (d, *J* = 7.7 Hz, 2H), 7.56 (t, *J* = 7.4 Hz, 1H), 7.44

(t, *J* = 7.6 Hz, 2H), 7.19 (d, *J* = 5.3 Hz, 1H), 5.18 (d, *J* = 8.1 Hz, 1H), 4.82 (dd, *J* = 12.8, 5.4 Hz, 1H), 4.13 – 3.99 (m, 1H), 3.92 (d, *J* = 15.0 Hz, 1H), 3.87 (d, *J* = 14.9 Hz, 1H), 3.67 (s, 3H), 3.01 (d, *J* = 5.2 Hz, 2H), 1.94 – 1.80 (m, 1H), 1.60 – 1.45 (m, 1H), 1.40 (s, 9H), 1.19 – 1.05 (m, 1H), 0.94 (d, *J* = 6.8 Hz, 3H), 0.88 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.7, 171.7, 170.8, 155.7, 135.1, 133.7, 128.8, 128.75, 79.8, 59.2, 52.6, 52.0, 37.7, 37.5, 34.1, 28.32, 24.7, 15.5, 11.6. HRMS (ESI) m/z Calculated for: $C_{23}H_{34}N_2NaO_6S^+$ $[M+Na]^+$ 489.2030, found 489.2027.



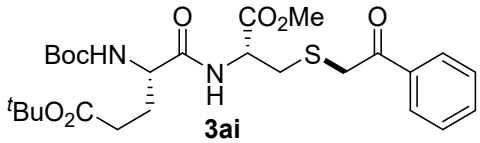
3ag

3ag: According to **General procedure I**, **3ag** was afforded as a yellow oil (54% yield). NMR showed the presence of at least two isomers. Major isomer: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.34 (d, *J* = 8.1 Hz, 1H), 7.98 (d, *J* = 7.5 Hz, 2H), 7.64 (t, *J* = 7.3 Hz, 1H), 7.52 (t, *J* = 7.6 Hz, 2H), 4.59 – 4.47 (m, 1H), 4.25 – 3.91 (m, 3H), 3.62 (s, 3H), 3.38 – 3.32 (m, 1H), 3.31 – 3.18 (m, 1H), 3.03 – 2.89 (m, 1H), 2.89 – 2.78 (m, 1H), 2.16 – 1.98 (m, 1H), 1.89 – 1.64 (m, 3H), 1.32 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 194.3, 172.8, 170.8, 155.5, 135.2, 135.1, 133.6, 128.7, 128.7, 80.6, 61.1, 52.6, 51.7, 47.0, 37.7, 34.3, 31.1, 28.3, 24.5. Minor isomer: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.32 (s, 1H), 7.98 (d, *J* = 7.5 Hz, 2H), 7.64 (t, *J* = 7.3 Hz, 1H), 7.52 (t, *J* = 7.6 Hz, 2H), 4.59 – 4.47 (m, 1H), 4.25 – 3.91 (m, 3H), 3.61 (s, 3H), 3.38 – 3.32 (m, 1H), 3.31 – 3.18 (m, 1H), 3.03 – 2.89 (m, 1H), 2.89 – 2.78 (m, 1H), 2.16 – 1.98 (m, 1H), 1.89 – 1.64 (m, 3H), 1.37 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 194.2, 172.2, 170.77, 154.6, 135.2, 135.1, 133.6, 128.7, 128.7, 80.3, 56.0, 52.6, 51.1, 47.0, 37.4, 34.3, 29.7, 28.3, 23.5.(containing a little EA). HRMS (ESI) m/z Calculated for: $C_{22}H_{30}N_2NaO_6S^+$ $[M+Na]^+$ 473.1717, found 473.1722.



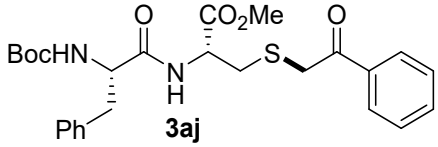
3ah

3ah: According to **General procedure I**, **3ah** was afforded as a white oil (56% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (d, *J* = 7.4 Hz, 2H), 7.55 (t, *J* = 7.4 Hz, 1H), 7.44 (t, *J* = 7.7 Hz, 2H), 7.36 (d, *J* = 6.8 Hz, 1H), 5.39 (d, *J* = 7.6 Hz, 1H), 4.80 (dd, *J* = 12.9, 5.5 Hz, 1H), 4.35 (d, *J* = 6.1 Hz, 1H), 3.91 (d, *J* = 14.9 Hz, 1H), 3.85 (d, *J* = 14.9 Hz, 1H), 3.67 (s, 3H), 3.01 (d, *J* = 4.6 Hz, 2H), 2.57 (t, *J* = 7.3 Hz, 2H), 2.16 – 2.02 (m, 4H), 1.99 – 1.84 (m, 1H), 1.40 (s, 9H).^{13}C NMR (100 MHz, $CDCl_3$) δ 194.7, 171.7, 170.7, 155.5, 135.0, 133.7, 128.8, 128.76, 80.0, 53.5, 52.7, 52.0, 37.7, 34.1, 31.9, 30.0, 28.3, 15.3. HRMS (ESI) m/z Calculated for: $C_{22}H_{32}N_2NaO_6S_2^+$ $[M+Na]^+$ 507.1594, found 507.1599.



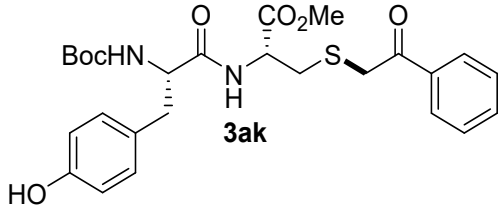
3ai

3ai: According to **General procedure I**, **3ai** was afforded as a yellow oil (46% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, *J* = 7.3 Hz, 2H), 7.59 (t, *J* = 7.4 Hz, 1H), 7.47 (t, *J* = 7.7 Hz, 2H), 7.00 (d, *J* = 6.8 Hz, 1H), 5.22 (d, *J* = 5.4 Hz, 1H), 4.88 – 4.78 (m, 1H), 4.22 (s, 1H), 3.93 (d, *J* = 14.8 Hz, 1H), 3.87 (d, *J* = 14.8 Hz, 1H), 3.71 (s, 3H), 3.08 (dd, *J* = 14.1, 4.5 Hz, 1H), 3.00 (dd, *J* = 14.0, 6.6 Hz, 1H), 2.35 (t, *J* = 7.4 Hz, 2H), 2.25 – 2.09 (m, 1H), 1.97 – 1.79 (m, 1H), 1.45 (s, 9H), 1.43 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 194.5, 172.1, 171.5, 171.1, 155.8, 135.1, 133.7, 128.8, 128.7, 82.1, 79.8, 53.5, 52.6, 51.8, 37.6, 34.2, 32.3, 29.7, 28.3, 28.0. HRMS (ESI) m/z Calculated for: $C_{26}H_{38}N_2NaO_8S^+$ $[M+Na]^+$ 561.2241, found 561.2243.



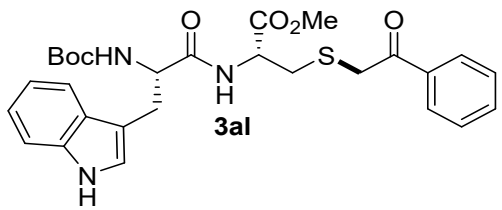
3aj

3aj: According to **General procedure I**, **3aj** was afforded as a yellow oil (52% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 – 7.86 (m, 2H), 7.58 (t, *J* = 7.4 Hz, 1H), 7.46 (t, *J* = 7.7 Hz, 2H), 7.30 – 7.23 (m, 2H), 7.23 – 7.17 (m, 3H), 7.12 (d, *J* = 7.4 Hz, 1H), 5.15 (d, *J* = 6.1 Hz, 1H), 4.80 (dd, *J* = 12.4, 5.4 Hz, 1H), 4.46 (s, 1H), 3.86 (d, *J* = 14.9 Hz, 1H), 3.78 (d, *J* = 14.9 Hz, 1H), 3.67 (s, 3H), 3.14 (dd, *J* = 13.9, 6.2 Hz, 1H), 3.09 – 3.01 (m, 1H), 3.01 – 2.92 (m, 2H), 1.38 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.7, 171.4, 170.6, 155.4, 136.6, 135.1, 133.7, 129.5, 128.8, 128.8, 128.6, 126.9, 80.1, 55.6, 52.7, 52.1, 38.3, 37.7, 34.3, 28.3. HRMS (ESI) m/z Calculated for: $C_{26}H_{32}N_2NaO_6S^+$ $[M+Na]^+$ 523.1873, found 523.1872.



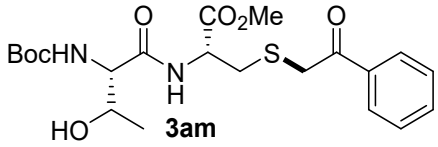
3ak

3ak: According to **General procedure I**, **3ak** was afforded as a yellow oil (42% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.18 (s, 1H), 8.45 (d, *J* = 7.8 Hz, 1H), 8.00 (d, *J* = 7.3 Hz, 2H), 7.64 (t, *J* = 7.4 Hz, 1H), 7.52 (t, *J* = 7.7 Hz, 2H), 7.04 (d, *J* = 8.4 Hz, 2H), 6.82 (d, *J* = 8.6 Hz, 1H), 6.65 (d, *J* = 8.3 Hz, 2H), 4.61 – 4.50 (m, 1H), 4.26 – 3.99 (m, 3H), 3.62 (s, 3H), 2.95 (dd, *J* = 13.8, 5.4 Hz, 1H), 2.90 – 2.78 (m, 2H), 2.68 – 2.55 (m, 1H), 1.29 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 195.3, 172.6, 171.4, 156.2, 155.7, 135.7, 133.9, 130.6, 129.2, 129.1, 128.5, 115.3, 78.5, 56.4, 52.6, 52.3, 38.3, 37.1, 33.0, 28.6. HRMS (ESI) m/z Calculated for: $C_{26}H_{32}N_2NaO_7S^+$ $[M+Na]^+$ 539.1822, found 539.1821.



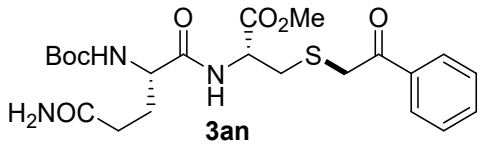
3al

3al: According to **General procedure I**, **3al** was afforded as a white oil (56% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (s, 1H), 7.94 – 7.85 (m, 2H), 7.65 (d, *J* = 7.7 Hz, 1H), 7.59 (t, *J* = 7.4 Hz, 1H), 7.46 (t, *J* = 7.7 Hz, 2H), 7.30 (d, *J* = 7.9 Hz, 1H), 7.22 – 7.06 (m, 3H), 7.00 (d, *J* = 5.4 Hz, 1H), 5.27 (d, *J* = 6.6 Hz, 1H), 4.77 (dd, *J* = 12.0, 5.2 Hz, 1H), 4.59 – 4.48 (m, 1H), 3.73 (d, *J* = 15.0 Hz, 1H), 3.63 (s, 3H), 3.57 (d, *J* = 14.9 Hz, 1H), 3.42 – 3.28 (m, 1H), 3.20 (dd, *J* = 14.5, 6.5 Hz, 1H), 2.98 – 2.75 (m, 2H), 1.42 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.8, 171.9, 170.5, 155.5, 136.3, 135.1, 133.8, 128.8, 128.7, 127.6, 123.7, 122.1, 119.6, 118.9, 111.3, 110.2, 80.1, 55.2, 52.7, 52.1, 37.8, 34.4, 31.6, 28.3. HRMS (ESI) m/z Calculated for: $C_{28}H_{34}N_3O_6S^+$ $[M+H]^+$ 540.2163, found 540.2164.



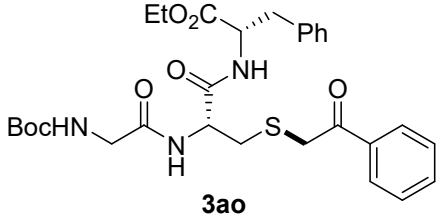
3am

3am: According to **General procedure I**, **3am** was afforded as a yellow oil (40% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (d, *J* = 7.6 Hz, 2H), 7.58 (t, *J* = 7.3 Hz, 1H), 7.51 (d, *J* = 7.3 Hz, 1H), 7.46 (t, *J* = 7.7 Hz, 2H), 5.55 (d, *J* = 7.8 Hz, 1H), 4.83 (q, *J* = 6.4 Hz, 1H), 4.44 (dd, *J* = 11.1, 5.7 Hz, 1H), 4.16 (d, *J* = 7.6 Hz, 1H), 3.89 (s, 2H), 3.72 (s, 3H), 3.09 (dd, *J* = 14.2, 4.2 Hz, 2H), 2.94 (dd, *J* = 14.3, 6.6 Hz, 1H), 1.44 (s, 9H), 1.20 (d, *J* = 6.3 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 194.7, 171.5, 170.7, 156.3, 134.9, 133.8, 128.8, 128.78, 80.3, 67.17, 58.9, 52.8, 52.1, 37.3, 33.7, 28.3, 18.4. HRMS (ESI) m/z Calculated for: $C_{21}H_{30}N_2NaO_7S^+$ $[M+Na]^+$ 477.1666, found 477.1669.



3an

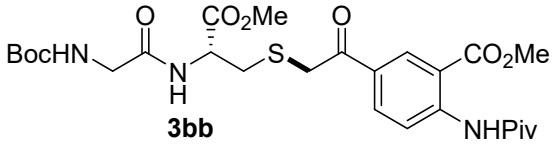
3an: According to **General procedure I**, **3an** was afforded as a white oil (62% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, *J* = 6.8 Hz, 1H), 7.93 (d, *J* = 7.7 Hz, 2H), 7.56 (t, *J* = 7.3 Hz, 1H), 7.44 (t, *J* = 7.6 Hz, 2H), 6.60 (s, 1H), 6.14 (s, 1H), 5.76 (d, *J* = 6.7 Hz, 1H), 4.85 – 4.75 (m, 1H), 4.32 – 4.21 (m, 1H), 4.02 – 3.80 (m, 2H), 3.68 (s, 3H), 3.05 (dd, *J* = 14.0, 4.2 Hz, 1H), 2.96 (dd, *J* = 14.0, 7.2 Hz, 1H), 2.36 (s, 2H), 2.14 – 1.85 (m, 2H), 1.39 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 194.8, 172.1, 171.2, 155.9, 135.1, 133.7, 128.8, 128.7, 80.0, 53.6, 52.7, 52.0, 37.8, 33.7, 29.7, 29.1, 28.3. HRMS (ESI) m/z Calculated for: $C_{22}H_{31}N_3NaO_7S^+$ $[M+Na]^+$ 504.1775, found 504.1774.



3ao

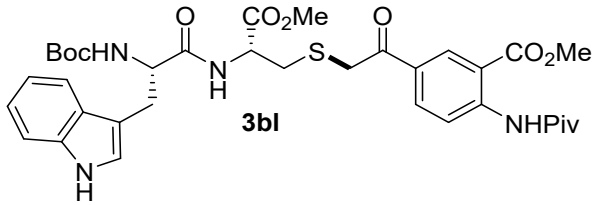
3ao: According to **General procedure I**, **3ao** was afforded as a white oil (61% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (d, *J* = 7.7 Hz, 2H), 7.57 (t, *J* = 7.3 Hz, 1H), 7.49

– 7.39 (m, 3H), 7.36 (d, *J* = 6.4 Hz, 1H), 7.24 – 7.12 (m, 4H), 5.32 (s, 1H), 4.75 (dd, *J* = 13.7, 7.0 Hz, 1H), 4.64 (dd, *J* = 13.5, 6.5 Hz, 1H), 4.22 – 4.06 (m, 2H), 4.01 (d, *J* = 15.9 Hz, 1H), 3.97 (d, *J* = 15.8 Hz, 1H), 3.85 – 3.69 (m, 2H), 3.14 (dd, *J* = 13.9, 5.9 Hz, 1H), 3.06 (dd, *J* = 13.8, 7.1 Hz, 1H), 2.94 (dd, *J* = 13.8, 5.9 Hz, 1H), 2.78 (dd, *J* = 14.3, 6.4 Hz, 1H), 1.42 (s, 9H), 1.17 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.4, 171.1, 169.8, 169.75, 136.0, 135.3, 133.8, 129.4, 128.8, 128.7, 128.5, 127.1, 80.2, 61.5, 53.9, 52.1, 44.1, 38.6, 37.8, 34.7, 28.3, 14.1. HRMS (ESI) m/z Calculated for: $C_{29}H_{37}N_3NaO_7S^+$ $[M+Na]^+$ 594.2244, found 594.2247.



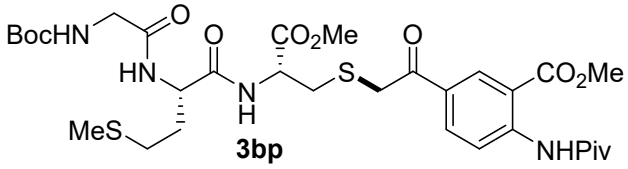
3bb

3bb: According to **General procedure I**, **3bb** was afforded as a white oil (30% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.59 (s, 1H), 8.89 (d, *J* = 9.0 Hz, 1H), 8.65 (d, *J* = 2.2 Hz, 1H), 8.09 (dd, *J* = 8.9, 2.1 Hz, 1H), 7.18 (d, *J* = 7.1 Hz, 1H), 5.24 (s, 1H), 4.85 (dd, *J* = 12.1, 5.9 Hz, 1H), 3.97 (s, 3H), 3.94 – 3.79 (m, 4H), 3.72 (s, 3H), 3.06 (dd, *J* = 14.2, 4.6 Hz, 1H), 2.99 (dd, *J* = 14.2, 6.1 Hz, 1H), 1.44 (s, 9H), 1.35 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 192.6, 178.4, 170.7, 169.9, 169.5, 168.2, 146.2, 134.9, 132.2, 128.6, 120.1, 114.7, 81.2, 58.5, 52.8, 40.7, 37.4, 34.1, 29.7, 28.3, 27.5, 18.5. HRMS (ESI) m/z Calculated for: $C_{26}H_{38}N_3O_9S^+$ $[M+H]^+$ 568.2323, found 568.2312.



3bl

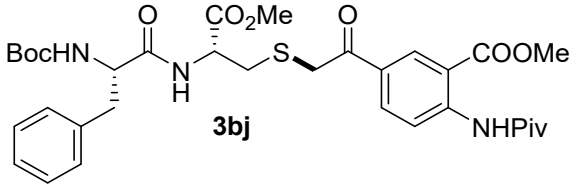
3bl: According to **General procedure I**, **3bl** was afforded as a yellow oil (35% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.58 (s, 1H), 8.86 (d, *J* = 8.9 Hz, 1H), 8.64 (d, *J* = 1.9 Hz, 1H), 8.06 (dd, *J* = 8.9, 1.8 Hz, 1H), 7.09 (d, *J* = 7.1 Hz, 1H), 7.04 (d, *J* = 8.0 Hz, 2H), 6.73 (d, *J* = 8.2 Hz, 2H), 5.17 (d, *J* = 6.9 Hz, 1H), 4.83 – 4.72 (m, 1H), 4.46 – 4.31 (m, 1H), 3.96 (s, 3H), 3.87 – 3.72 (m, 2H), 3.69 (s, 3H), 3.07 – 2.92 (m, 4H), 1.40 (s, 9H), 1.35 (s, 9H). ^{13}C NMR (100 MHz, CDCl3) δ 192.9, 178.6, 171.7, 170.5, 168.2, 155.5, 155.3, 146.0, 134.9, 132.2, 130.6, 128.7, 127.9, 120.2, 115.67, 114.9, 80.3, 52.8, 52.81, 52.2, 40.7, 37.4, 34.3, 29.7, 28.3, 27.5, 14.2. HRMS (ESI) m/z Calculated for: $C_{35}H_{45}N_4O_9S^+$ $[M+H]^+$ 697.2902, found 697.2908.



3bp

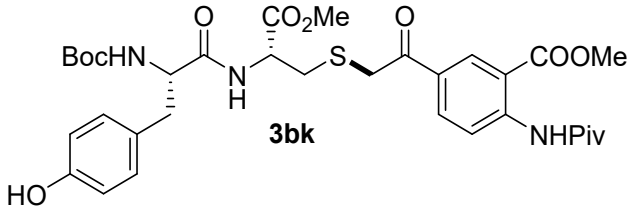
3bp: According to **General procedure I**, **3bp** was afforded as a yellow oil (40% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.58 (s, 1H), 8.86 (d, *J* = 8.9 Hz, 1H), 8.64 (d, *J* = 2.1 Hz, 1H), 8.08 (dd, *J* = 8.9, 2.0 Hz, 1H), 7.56 (d, *J* = 7.8 Hz, 1H), 7.14 (d, *J* = 7.6 Hz, 1H), 5.41 (t, *J* = 4.9 Hz, 1H), 4.83 – 4.67 (m, 2H), 3.96 (s, 3H), 3.88 (s, 2H),

3.84 (d, *J* = 5.1 Hz, 1H), 3.80 (d, *J* = 4.7 Hz, 1H), 3.71 (s, 3H), 3.06 – 2.92 (m, 2H), 2.58 (t, *J* = 7.2 Hz, 2H), 2.15 – 2.10 (m, 1H), 2.09 (s, 3H), 2.05 – 1.99 (m, 1H), 1.41 (s, 9H), 1.33 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 193.0, 178.4, 171.1, 170.6, 169.8, 168.2, 146.2, 134.9, 132.3, 128.5, 120.0, 114.8, 80.3, 59.4, 52.8, 52.2, 52.1, 44.4, 40.7, 37.2, 33.6, 31.3, 29.8, 28.3, 27.5, 15.2. HRMS (ESI) m/z Calculated for: $C_{31}H_{46}N_4NaO_{10}S_2^+$ $[M+Na]^+$ 721.2548, found 721.2549.



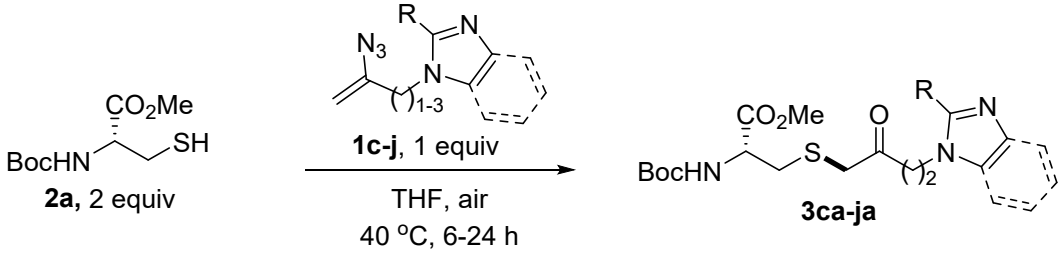
3bj

3bj: According to **General procedure I**, **3bj** was afforded as a white oil (50% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.58 (s, 1H), 8.89 (d, *J* = 9.0 Hz, 1H), 8.64 (d, *J* = 2.2 Hz, 1H), 8.27 – 7.88 (m, 1H), 7.29 – 7.26 (m, 1H), 7.23 – 7.18 (m, 3H), 7.08 (d, *J* = 7.6 Hz, 1H), 5.13 (d, *J* = 6.7 Hz, 1H), 4.80 (dd, *J* = 12.2, 5.7 Hz, 1H), 4.45 (d, *J* = 6.1 Hz, 1H), 3.96 (s, 3H), 3.83 (d, *J* = 14.7 Hz, 1H), 3.74 (d, *J* = 14.7 Hz, 1H), 3.69 (s, 3H), 3.13 (dd, *J* = 13.9, 6.2 Hz, 1H), 3.09 – 3.02 (m, 1H), 3.02 – 2.91 (m, 2H), 1.38 (s, 9H), 1.35 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 192.7, 178.4, 171.4, 170.5, 168.2, 155.4, 146.2, 136.6, 134.9, 132.2, 129.4, 128.6, 126.9, 120.0, 114.7, 80.2, 55.7, 52.8, 52.7, 52.1, 40.7, 38.3, 37.2, 34.2, 28.3, 27.5. HRMS (ESI) m/z Calculated for: $C_{33}H_{43}N_3NaO_9S^+$ $[M+Na]^+$ 680.2612, found 680.2610.



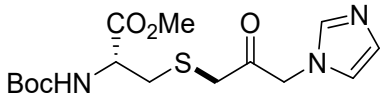
3bk

3bk: According to **General procedure I**, **3bk** was afforded as a white oil (61% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.59 (s, 1H), 8.84 (d, *J* = 8.9 Hz, 1H), 8.63 (d, *J* = 2.1 Hz, 1H), 8.05 (dd, *J* = 9.0, 2.1 Hz, 1H), 7.16 (d, *J* = 7.3 Hz, 1H), 7.02 (d, *J* = 8.2 Hz, 2H), 6.72 (d, *J* = 8.4 Hz, 2H), 5.23 (d, *J* = 7.3 Hz, 1H), 4.78 (dd, *J* = 12.2, 5.4 Hz, 1H), 4.39 (d, *J* = 6.4 Hz, 1H), 3.95 (s, 3H), 3.88 – 3.71 (m, 2H), 3.67 (s, 3H), 3.10 – 2.80 (m, 4H), 1.39 (s, 9H), 1.34 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.9, 177.6, 170.8, 169.5, 167.1, 154.5, 145.0, 133.8, 131.2, 129.5, 127.6, 126.5, 119.1, 114.6, 113.8, 98.9, 79.3, 54.8, 51.8, 51.7, 51.1,39.6, 36.2, 33.1, 28.7, 27.2, 26.4. HRMS (ESI) m/z Calculated for: $C_{33}H_{44}N_3O_{10}S^+$ $[M+H]^+$ 674.2742, found 674.2754.



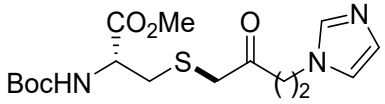
General procedure II: To a 10 mL round-bottom flask, were added peptide **2a** (0.4

mmol, 2 equiv), THF (3mL) and vinyl azide **1c-j** (0.2 mmol, 1 equiv). The reaction mixture was stirred at 40 °C in the air atmosphere until the peptide was consumed (monitored by TLC). Then it was cooled to room temperature, concentrated and purified by flash column chromatography on silica gel (MeOH/DCM) to provide product **3ca-ja**.



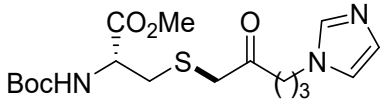
3ca

3ca: According to **General procedure II**, **3ca** was afforded as a yellow oil (78% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 (s, 1H), 7.11 (s, 1H), 6.93 (s, 1H), 5.35 (d, *J* = 7.2 Hz, 1H), 4.98 (s, 2H), 4.51 (d, *J* = 5.3 Hz, 1H), 3.75 (s, 3H), 3.35 – 3.19 (m, 2H), 3.01 (dd, *J* = 14.0, 4.7 Hz, 1H), 2.85 (dd, *J* = 14.1, 6.3 Hz, 1H), 1.44 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 198.0, 171.2, 155.2, 138.1, 129.8, 120.0, 80.5, 53.1, 53.0, 38.1, 34.9, 29.7, 28.3. HRMS (ESI) m/z Calculated for: $C_{15}H_{24}N_3O_5S^+$ $[M+H]^+$ 358.1431, found 358.1434.



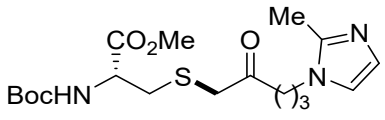
3da

3da: According to **General procedure II**, **3da** was afforded as a yellow oil (86% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (s, 1H), 6.94 (s, 1H), 6.86 (s, 1H), 5.48 (s, 1H), 4.39 (d, *J* = 4.8 Hz, 1H), 4.20 (t, *J* = 5.6 Hz, 2H), 3.69 (s, 3H), 3.25 – 3.08 (m, 2H), 3.02 (t, *J* = 6.2 Hz, 2H), 2.79 (dd, *J* = 13.7, 3.9 Hz, 1H), 2.65 (dd, *J* = 13.8, 6.5 Hz, 1H), 1.38 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.1, 171.3, 155.2, 137.4, 129.3, 119.1, 80.2, 52.9, 52.7, 41.2, 41.1, 34.26, 29.6, 28.3. HRMS (ESI) m/z Calculated for: $C_{16}H_{26}N_3O_5S^+$ $[M+H]^+$ 372.1588, found 372.1590.



3ea

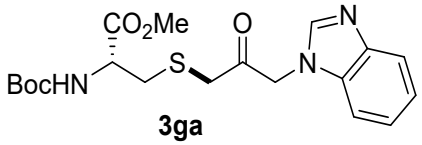
3ea: According to **General procedure II**, **3ea** was afforded as a yellow oil (90% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (s, 1H), 6.94 (s, 1H), 6.82 (s, 1H), 5.62 (d, *J* = 7.6 Hz, 1H), 4.39 (d, *J* = 5.6 Hz, 1H), 3.88 (t, *J* = 6.9 Hz, 2H), 3.65 (s, 3H), 3.26 – 3.11 (m, 2H), 2.87 (dd, *J* = 13.8, 4.5 Hz, 1H), 2.76 (dd, *J* = 13.8, 6.4 Hz, 1H), 2.46 (t, *J* = 6.8 Hz, 2H), 2.06 – 1.82 (m, 2H), 1.34 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 204.0, 171.3, 155.2, 137.1, 129.5, 118.7, 80.1, 53.0, 52.6, 45.7, 41.1, 36.6, 34.4, 28.3, 24.9. HRMS (ESI) m/z Calculated for: $C_{17}H_{27}N_3NaO_5S^+$ $[M+Na]^+$ 408.1564, found 408.1568.



3fa

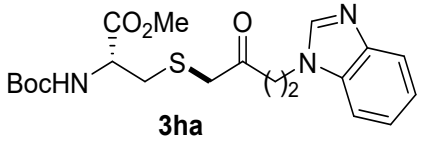
3fa: According to **General procedure II**, **3fa** was afforded as a yellow oil (80%

yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.84 (s, 1H), 6.76 (s, 1H), 5.48 (d, *J* = 7.5 Hz, 1H), 4.46 (d, *J* = 5.9 Hz, 1H), 3.82 (t, *J* = 7.2 Hz, 2H), 3.71 (s, 3H), 3.27 – 3.15 (m, 2H), 2.93 (dd, *J* = 13.9, 4.7 Hz, 1H), 2.81 (dd, *J* = 13.9, 6.3 Hz, 1H), 2.54 (t, *J* = 6.8 Hz, 2H), 2.32 (s, 3H), 2.04 – 1.88 (m, 2H), 1.39 (s, 9H).^{13}C NMR (100 MHz, $CDCl_3$) δ 204.0, 171.3, 155.2, 144.4, 127.2, 119.0, 80.3, 53.0, 52.7, 44.8, 41.2, 36.7, 34.6, 28.3, 24.5, 13.0. HRMS (ESI) m/z Calculated for: $C_{18}H_{30}N_3O_5S^+$ $[M+H]^+$ 400.1901, found 400.1904.



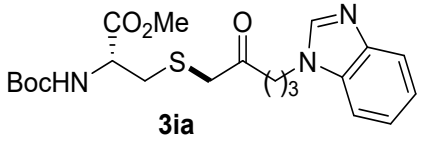
3ga

3ga: According to **General procedure II**, **3ga** was afforded as a yellow oil (90% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (s, 1H), 7.86 – 7.78 (m, 1H), 7.35 – 7.26 (m, 3H), 5.36 (d, *J* = 4.9 Hz, 1H), 5.16 (s, 2H), 4.58 – 4.43 (m, 1H), 3.72 (s, 3H), 3.34 – 3.18 (m, 2H), 3.01 (dd, *J* = 14.0, 4.6 Hz, 1H), 2.84 (dd, *J* = 14.0, 6.3 Hz, 1H), 1.44 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 198.0, 171.2, 143.7, 143.6, 133.9, 123.6, 122.7, 120.7, 109.1, 100.0, 80.5, 52.8, 51.0, 38.0, 34.93, 34.9, 28.3. HRMS (ESI) m/z Calculated for: $C_{19}H_{26}N_3O_5S^+$ $[M+H]^+$ 408.1588, found 408.1590.



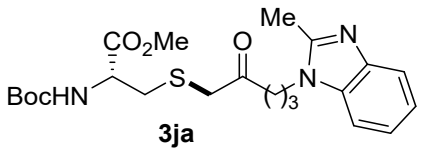
3ha

3ha: According to **General procedure II, 3ha** was afforded as a white oil (99% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (s, 1H), 7.76 (d, *J* = 7.4 Hz, 1H), 7.37 (d, *J* = 7.8 Hz, 1H), 7.31 – 7.17 (m, 2H), 5.36 (d, *J* = 7.7 Hz, 1H), 4.55 – 4.43 (m, 2H), 4.42 – 4.37 (m, 1H), 3.70 (s, 3H), 3.28 – 3.02 (m, 4H), 2.78 (dd, *J* = 13.9, 4.4 Hz, 1H), 2.62 (dd, *J* = 13.8, 6.5 Hz, 1H), 1.41 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.2, 171.2, 155.2, 143.8, 143.6, 133.3, 123.0, 122.2, 120.5, 109.4, 80.3, 52.9, 52.7, 41.3, 39.7, 39.2, 34.4, 28.3. HRMS (ESI) m/z Calculated for: $C_{20}H_{28}N_3O_5S^+$ $[M+H]^+$ 422.1744, found 422.1747.



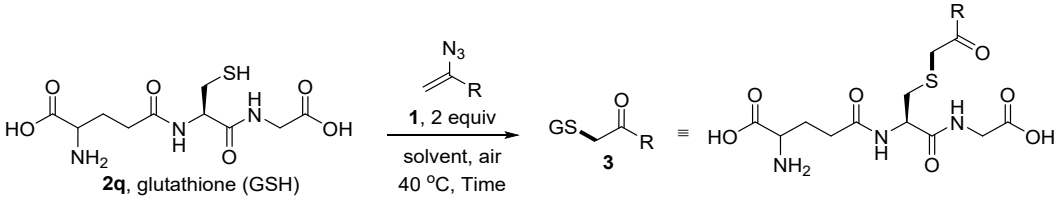
3ia

3ia: According to **General procedure II**, **3ia** was afforded as a yellow oil (99% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (s, 1H), 7.78 – 7.69 (m, 1H), 7.43 – 7.32 (m, 1H), 7.26 – 7.15 (m, 2H), 5.56 (d, *J* = 7.9 Hz, 1H), 4.54 – 4.33 (m, 1H), 4.14 (t, *J* = 7.1 Hz, 2H), 3.68 (s, 3H), 3.36 – 3.09 (m, 2H), 2.91 (dd, *J* = 13.9, 4.8 Hz, 1H), 2.80 (dd, *J* = 13.8, 6.4 Hz, 1H), 2.52 (t, *J* = 6.7 Hz, 2H), 2.15 – 1.98 (m, 2H), 1.38 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 204.1, 171.3, 155.2, 143.8, 143.0, 133.6, 123.0, 122.2, 120.3, 109.8, 80.2, 53.1, 52.7, 43.8, 41.2, 36.7, 34.5, 28.3, 23.7. HRMS (ESI) m/z Calculated for: $C_{21}H_{30}N_3O_5S^+$ $[M+H]^+$ 436.1901, found 436.1904.

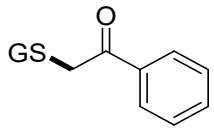


3ja

3ja: According to **General procedure II**, **3ja** was afforded as a yellow oil (99% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 – 7.58 (m, 1H), 7.33 – 7.27 (m, 1H), 7.22 – 7.15 (m, 2H), 5.43 (d, *J* = 7.7 Hz, 1H), 4.67 – 4.35 (m, 1H), 4.08 (t, *J* = 7.4 Hz, 2H), 3.71 (s, 3H), 3.31 – 3.13 (m, 2H), 2.94 (dd, *J* = 13.9, 4.7 Hz, 1H), 2.82 (dd, *J* = 13.9, 6.2 Hz, 1H), 2.65 – 2.51 (m, 5H), 2.09 – 1.99 (m, 2H), 1.40 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 204.2, 171.3, 155.2, 151.4, 142.6, 135.1, 122.1, 121.9, 119.1, 109.2, 80.3, 53.0, 52.7, 42.6, 41.3, 36.8, 34.6, 28.3, 23.4, 13.9. HRMS (ESI) m/z Calculated for: $C_{22}H_{32}N_3O_5S^+$ $[M+H]^+$ 450.2057, found 450.2062.

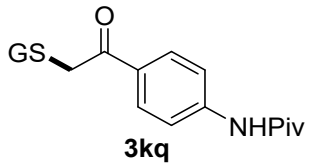


General procedure III: To a 10 mL round-bottom flask, were added peptide **2q** (0.2 mmol, 1 equiv) in 2 mL PBS (pH 7.4) and vinyl azide 1 (0.4 mmol, 2 equiv) in 2 mL THF. The reaction mixture was stirred at 40 °C in the air atmosphere until the peptide was consumed (monitored by TLC, n-BuOH/AcOH/H_2O=3/1/1). After the reaction was complete, the solvent was spun off directly. The crude product was purified by preparative HPLC (Method C) and concentrated by lyophilization.



3aq

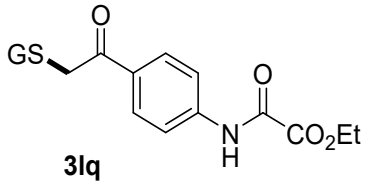
3aq: According to **General procedure III**, **3aq** was afforded as a white solid (75% yield). ^{1}H NMR (500 MHz, D_2O) δ 7.85 (d, *J* = 7.9 Hz, 2H), 7.56 (t, *J* = 7.2 Hz, 1H), 7.42 (t, *J* = 7.5 Hz, 2H), 4.43 (dd, *J* = 8.8, 4.8 Hz, 1H), 4.01 – 3.92 (m, 1H), 3.81 – 3.36 (m, 4H), 2.96 (dd, *J* = 14.2, 4.7 Hz, 1H), 2.76 (dd, *J* = 14.2, 9.0 Hz, 1H), 2.32 (t, *J* = 7.6 Hz, 2H), 1.96 (dd, *J* = 14.5, 7.0 Hz, 2H). ^{13}C NMR (75 MHz, D_2O) δ 198.7, 175.3, 174.8, 173.8, 171.8, 134.6, 134.5, 129.0, 128.8, 54.0, 52.8, 42.8, 38.7, 33.2, 31.3, 26.1.HRMS (ESI) m/z Calculated for: $C_{18}H_{24}N_3O_7S^+$ $[M+H]^+$ 426.1329, found 426.1320.



3kq

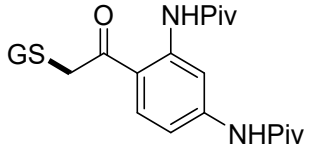
3kq: According to **General procedure III**, **3kq** was afforded as a white solid (70% yield). ^{1}H NMR (400 MHz, D_2O) δ 7.59 (d, *J* = 8.7 Hz, 2H), 7.28 (d, *J* = 8.7 Hz, 2H), 4.36 (dd, *J* = 9.0, 4.8 Hz, 1H), 3.80 – 3.70 (m, 2H), 3.61 – 3.51 (m, 3H), 2.84 (dd, *J* =

14.1, 4.8 Hz, 1H), 2.65 (dd, *J* = 14.1, 9.1 Hz, 1H), 2.28 (t, *J* = 7.7 Hz, 2H), 1.98 – 1.87 (m, 2H), 1.04 (s, 9H).^{13}C NMR (100 MHz, D_2O) δ 196.9, 180.9, 176.1, 174.8, 173.9, 171.6, 142.8, 130.5, 130.0, 121.1, 54.1, 52.8, 52.6, 43.4, 39.4, 33.3, 31.4, 26.5, 26.2. HRMS (ESI) m/z Calculated for: $C_{23}H_{31}N_4O_8S^-$ $[M-H]^-$ 523.1868, found 523.1863.



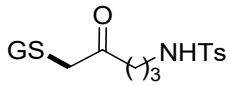
3lq

3lq: According to **General procedure III**, **3lp** was afforded as a white solid (75% yield). ^{1}H NMR (500 MHz, D_2O) δ 8.79 – 8.60 (m, 2H), 8.50 – 8.30 (m, 2H), 5.28 (dd, *J* = 8.9, 4.8 Hz, 1H), 5.12 (q, *J* = 7.1 Hz, 2H), 4.58 – 4.33 (m, 4H), 3.81 (dd, *J* = 14.2, 4.7 Hz, 1H), 3.61 (dd, *J* = 14.2, 9.0 Hz, 1H), 3.34 – 3.03 (m, 3H), 2.88 – 2.75 (m, 3H), 2.09 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, D_2O) δ 176.1, 175.0, 174.9, 174.0, 171.9, 171.5, 145.2, 134.7, 130.0, 126.9, 54.1, 52.5, 49.6, 43.3, 38.7, 32.9, 31.4, 26.2, 20.7. HRMS (ESI) m/z Calculated for: $C_{22}H_{27}N_4O_{10}S^-$ $[M-H]^-$ 539.1453, found 539.1494.



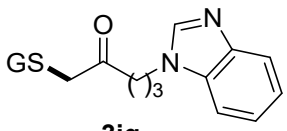
3mq

3mq: According to **General procedure III**, **3mq** was afforded as a white solid (73% yield). ^{1}H NMR (400 MHz, D_2O) δ 8.40 (s, 1H), 7.68 (d, *J* = 8.8 Hz, 1H), 7.19 (d, *J* = 8.7 Hz, 1H), 4.49 – 4.42 (m, 1H), 3.65 – 3.54 (m, 3H), 2.84 – 2.66 (m, 2H), 2.40 – 2.28 (m, 2H), 1.97 (dd, *J* = 14.1, 7.3 Hz, 2H), 1.29 – 1.23 (m, 1H), 1.19 – 1.15 (m, 1H), 1.15 – 0.95 (m, 18H). HRMS (ESI) m/z Calculated for: $C_{28}H_{40}N_5O_9S^-$ $[M-H]^-$ 622.2552, found 622.2548.



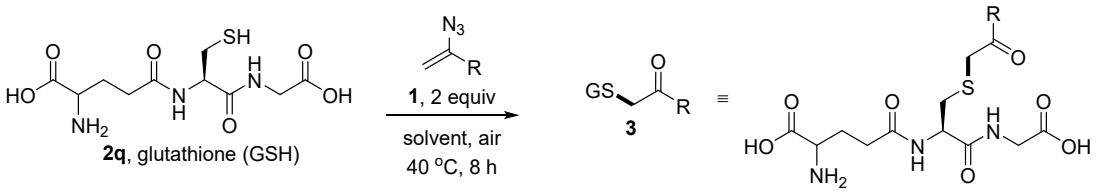
3nq

3nq: According to **General procedure III**, **3nq** was afforded as a white solid (80% yield). ^{1}H NMR (400 MHz, D_2O) δ 7.66 (d, *J* = 8.3 Hz, 2H), 7.37 (d, *J* = 8.1 Hz, 2H), 4.48 (dd, *J* = 8.9, 4.9 Hz, 1H), 3.80 – 3.64 (m, 2H), 3.48 (t, *J* = 6.4 Hz, 1H), 2.91 (dd, *J* = 14.1, 4.9 Hz, 1H), 2.83 (t, *J* = 6.7 Hz, 2H), 2.75 (dd, *J* = 14.1, 9.0 Hz, 1H), 2.55 (t, *J* = 7.0 Hz, 2H), 2.47 – 2.38 (m, 2H), 2.35 (s, 3H), 2.04 – 1.89 (m, 2H), 1.70 – 1.55 (m, 2H).^{13}C NMR (75 MHz, D_2O) δ 209.2, 175.5, 174.8 – 172.3 (m), 171.7, 144.4, 135.5, 130.0, 126.7, 54.1, 52.8, 43.0, 41.9, 41.7, 37.6, 33.1, 31.4, 26.2, 23.0, 20.8. HRMS (ESI) m/z Calculated for: $C_{22}H_{32}N_4NaO_9S_2^+$ $[M+Na]^+$ 583.1503, found 583.1499.

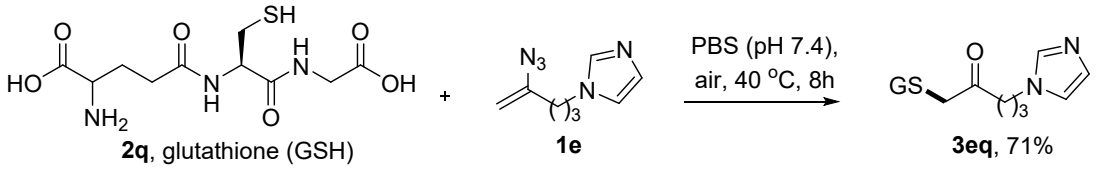


3iq

3iq: According to **General procedure III**, **3iq** was afforded as a yellow solid (78% yield). ^{1}H NMR (400 MHz, D_2O) δ 8.83 (s, 1H), 7.69 – 7.53 (m, 2H), 7.42 – 7.35 (m, 2H), 4.36 (dd, *J* = 8.5, 5.0 Hz, 1H), 4.29 – 4.20 (m, 2H), 3.69 – 3.58 (m, 3H), 3.41 – 3.14 (m, 2H), 2.72 (dd, *J* = 14.1, 4.9 Hz, 1H), 2.67 – 2.53 (m, 3H), 2.39 (t, *J* = 7.6 Hz, 2H), 2.07 – 1.97 (m, 4H).^{13}C NMR (100 MHz, D_2O) δ 208.4, 176.0, 174.8, 173.9, 171.6, 140.6, 132.8, 131.2, 126.0, 125.8, 115.4, 112.17, 100.0, 54.0, 52.7, 45.6, 43.2, 37.3, 33.1, 33.0, 31.3, 26.1, 22.6. HRMS (ESI) m/z Calculated for: $C_{22}H_{30}N_5O_7S^+$ $[M+H]^+$ 508.1860, found 508.1859.



General procedure IV: To a 5 mL round-bottom flask, were added peptide **2q** (0.2 mmol), 2 mL PBS buffer (10 mmol/L, pH 7.4) and α-vinyl azide (**1**, 2 equiv). The mixed solution was stired at 40 °C for 8 h in the air atomsphere. Then the reaction was analyzed by LC-MS to give LC-MS yield.



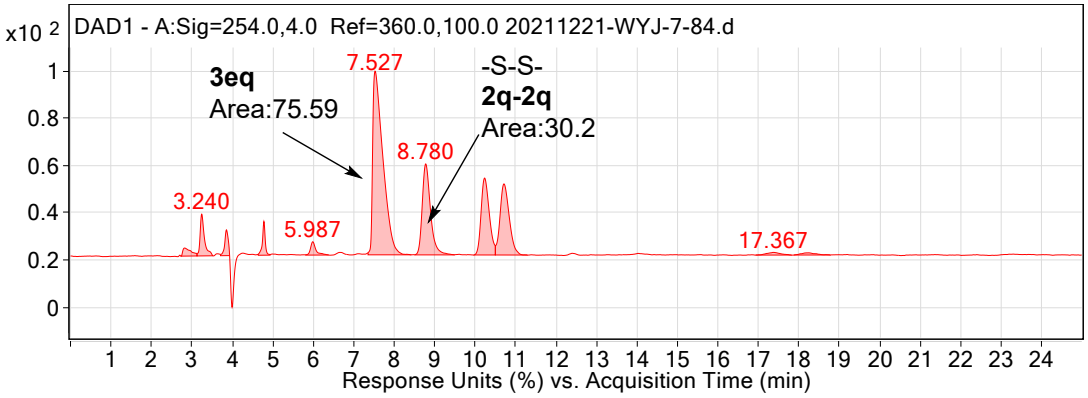


Figure S1. LC-MS TIC curve of **3eq**. Gradient used：Method B

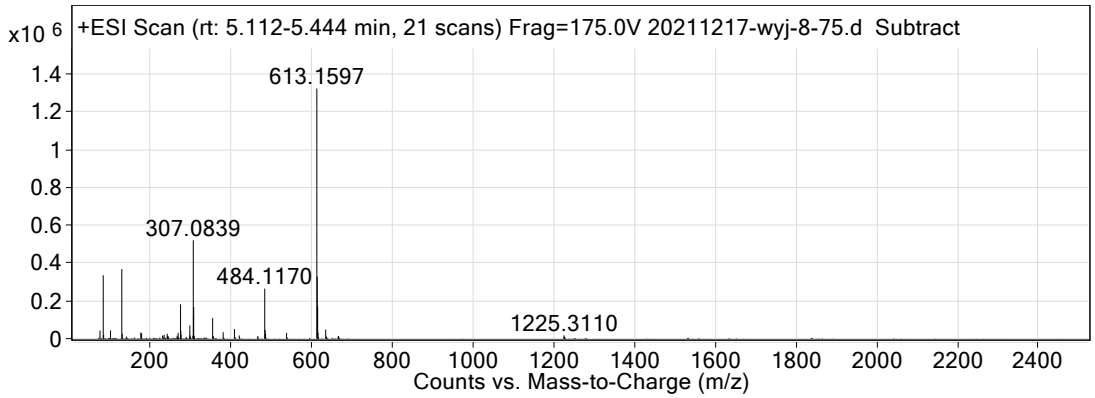


Figure S2. ESI Mass spectrum of dimerized by product by peptide **2q**. Calculated Mass $[M+H]^+$ 613.1592, $[M+2H]^{2+}$ 307.0833; Mass Found (ESI+) $[M+H]^+$ 613.1597, $[M+2H]^{2+}$ 307.0839.

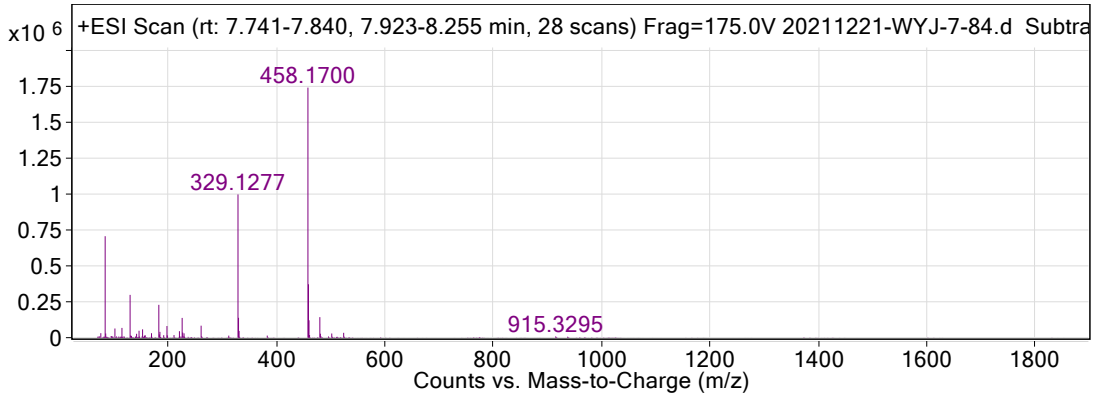
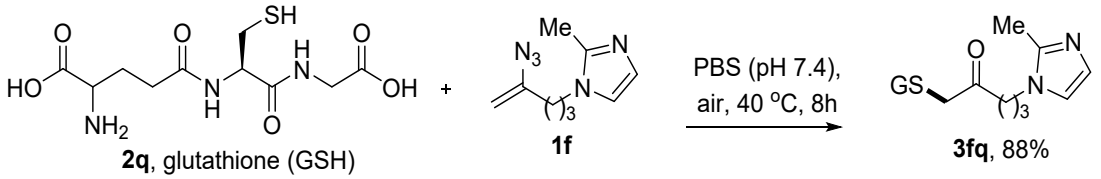


Figure S3. ESI Mass spectrum of modified product **3eq**. Calculated Mass $[M+H]^+$ 458.1704, $[M-C_5H_7NO_3]^+$ 329.1278; Mass Found (ESI+) $[M+H]^+$ 458.1700, $[M-C_5H_7NO_3]^+$ 329.1277.



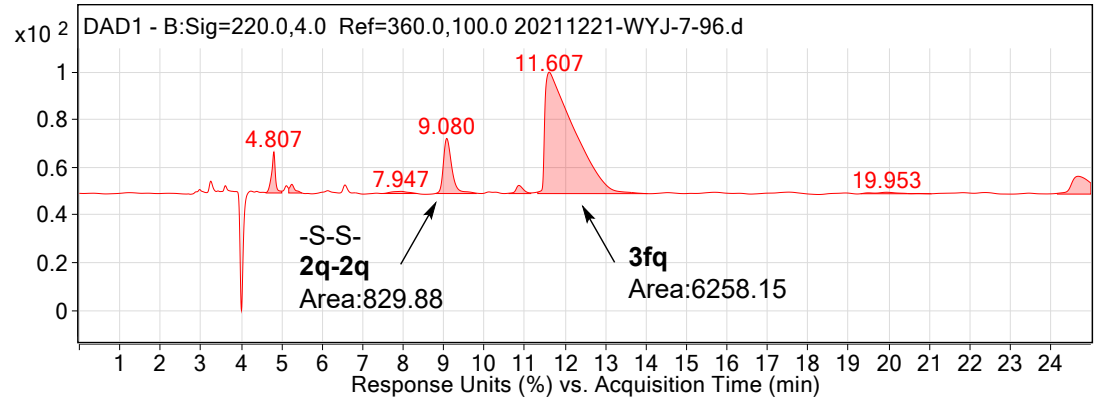


Figure S4. LC-MS TIC curve of **3fq**. Gradient used：Method B

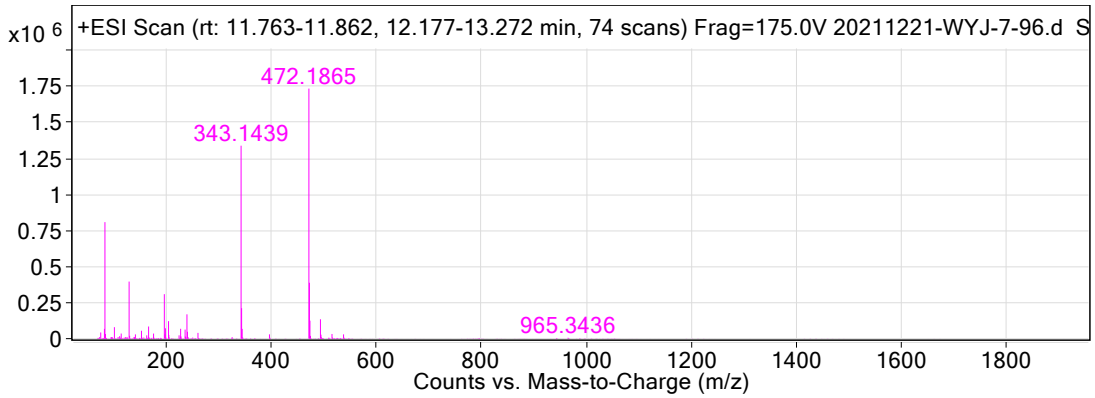


Figure S5. ESI Mass spectrum of modified product **3fq**. Calculated Mass $[M+H]^+$ 472.1860, $[M\text{-}C_5H_7NO_3]^+$ 343.1435; Mass Found (ESI+) $[M+H]^+$ 472.1865, $[M\text{-}C_5H_7NO_3]^+$ 343.1439.

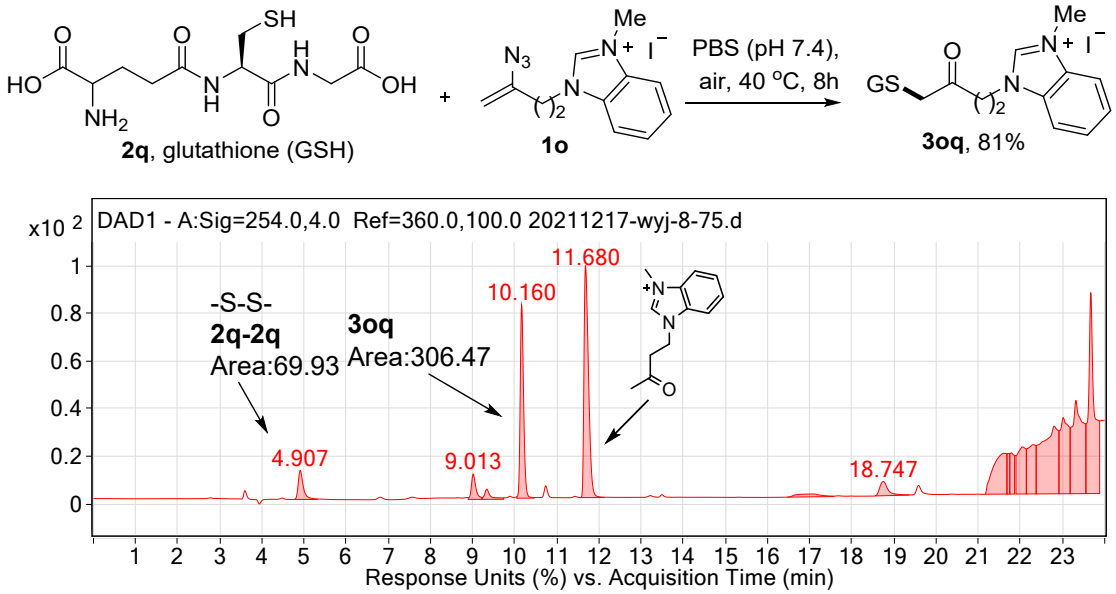


Figure S6. LC-MS TIC curve of **3oq**. Gradient used：Method A

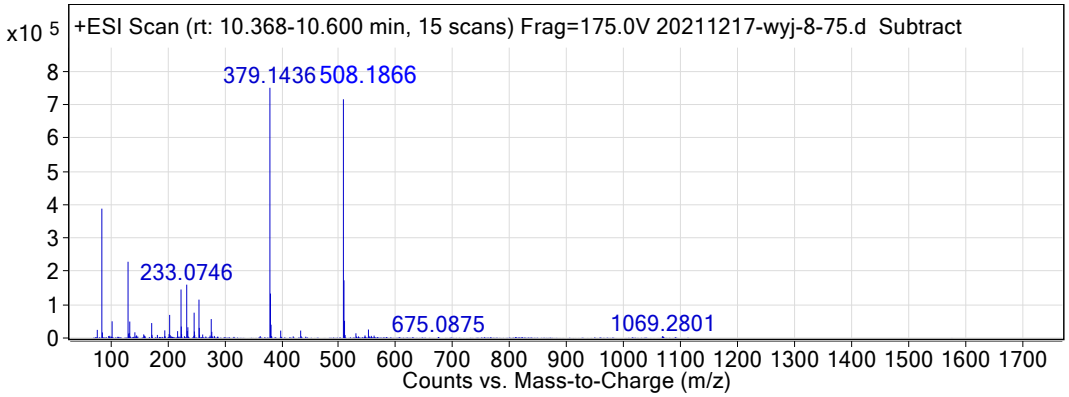


Figure S7. ESI Mass spectrum of modified product **3oq**. Calculated Mass $[M+H]^+$ 508.1860, $[M\text{-}C_5H_7NO_3]^+$ 379.1435; Mass Found (ESI+) $[M+H]^+$ 508.1866, $[M\text{-}C_5H_7NO_3]^+$ 379.1436.

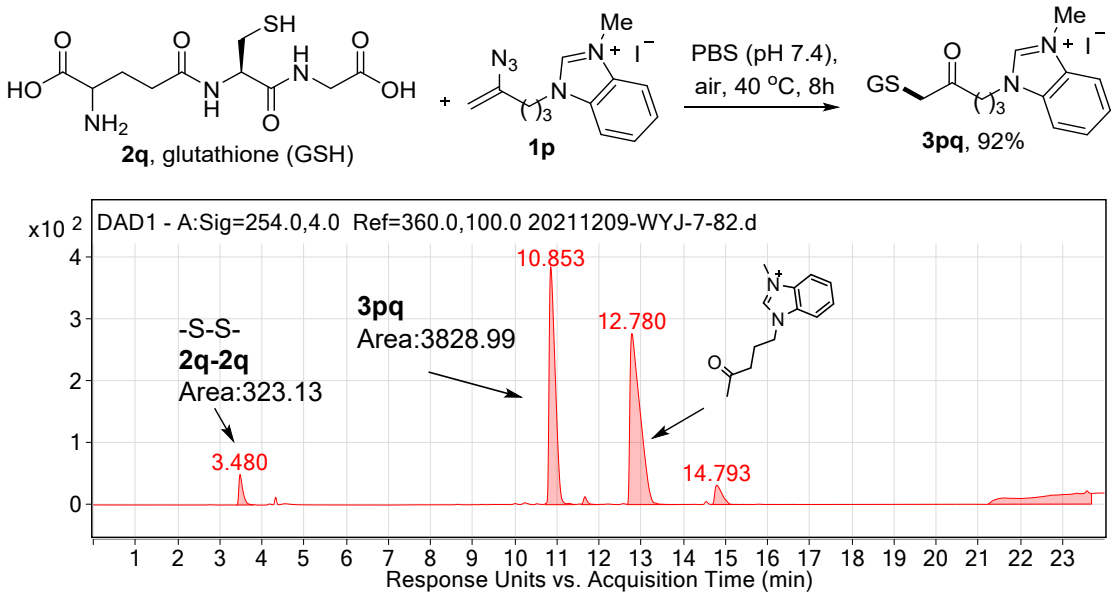


Figure S8. LC-MS TIC curve of **3pq**. Gradient used：Method A

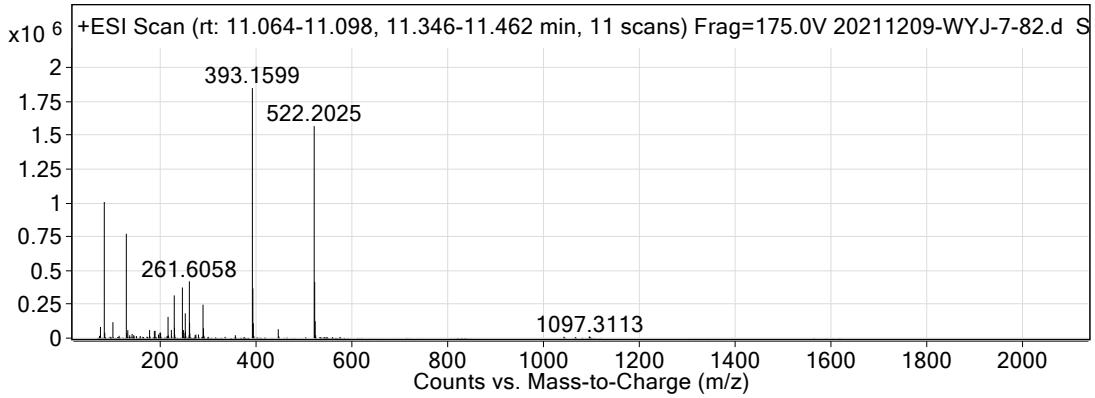
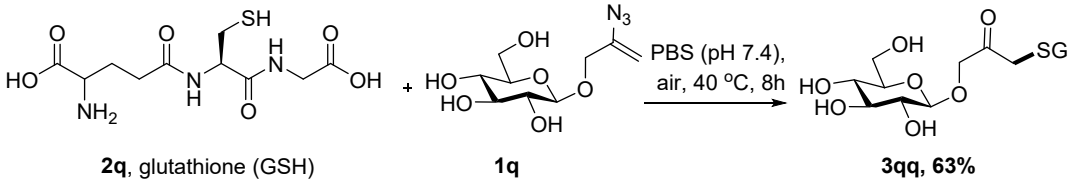


Figure S9. ESI Mass spectrum of modified product **3pq**. Calculated Mass $[M+H]^+$ 522.2017, $[M-C_5H_7NO_3]^+$ 393.1591; Mass Found (ESI+) $[M+H]^+$ 522.2025, $[M-C_5H_7NO_3]^+$ 393.1599.



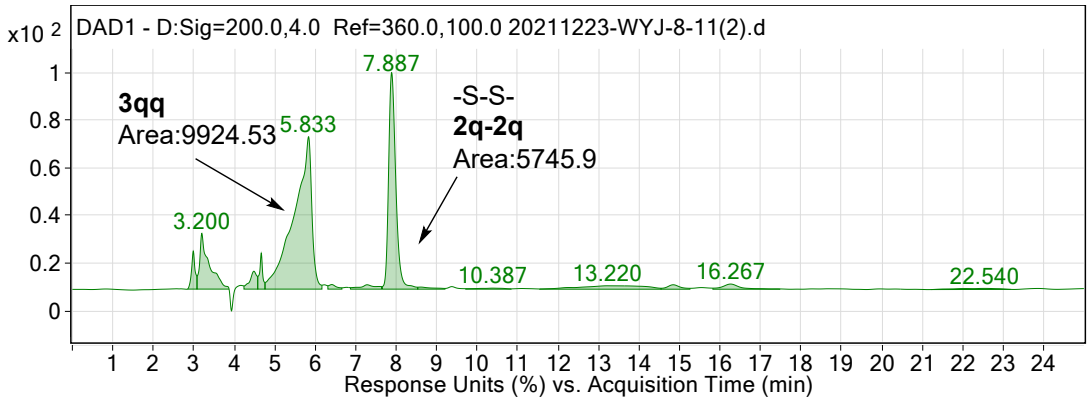


Figure S10. LC-MS TIC curve of **3qq**. Gradient used：Method B

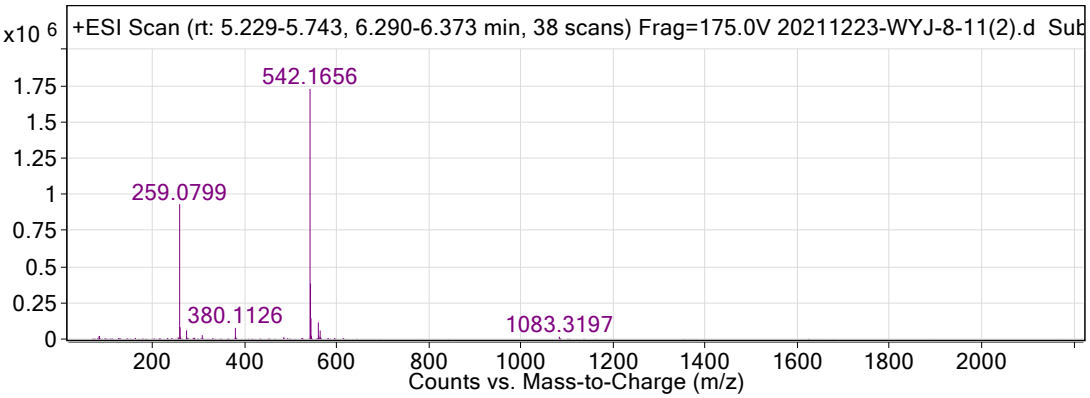
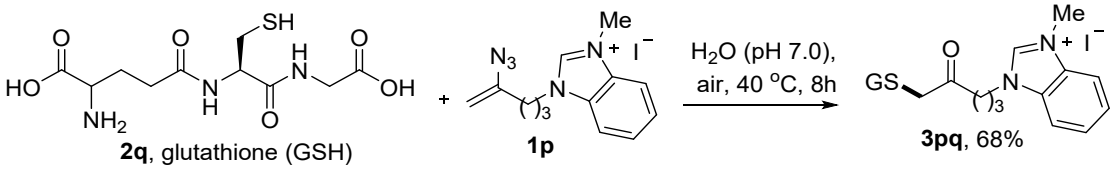


Figure S11. ESI Mass spectrum of modified product **3qq**. Calculated Mass $[M+H]^+$ 542.1650; Mass Found (ESI+) $[M+H]^+$ 542.1656.



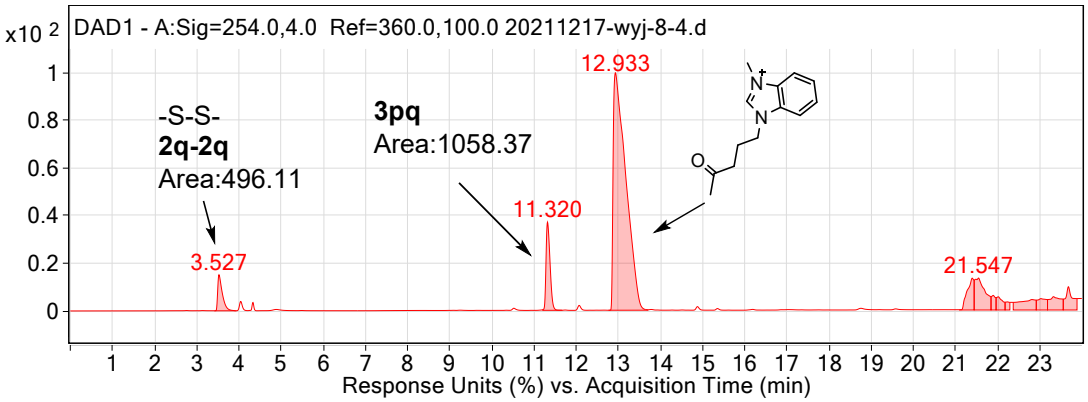
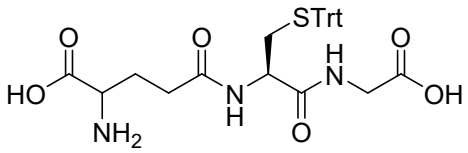


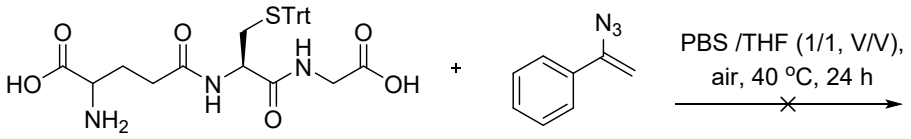
Figure S12. ESI Mass spectrum of modified product **3pq**. Calculated Mass $[M+H]^+$ 542.1650; Mass Found (ESI+) $[M+H]^+$ 542.1656.

5. Control reaction of Trt-GSH with 1a



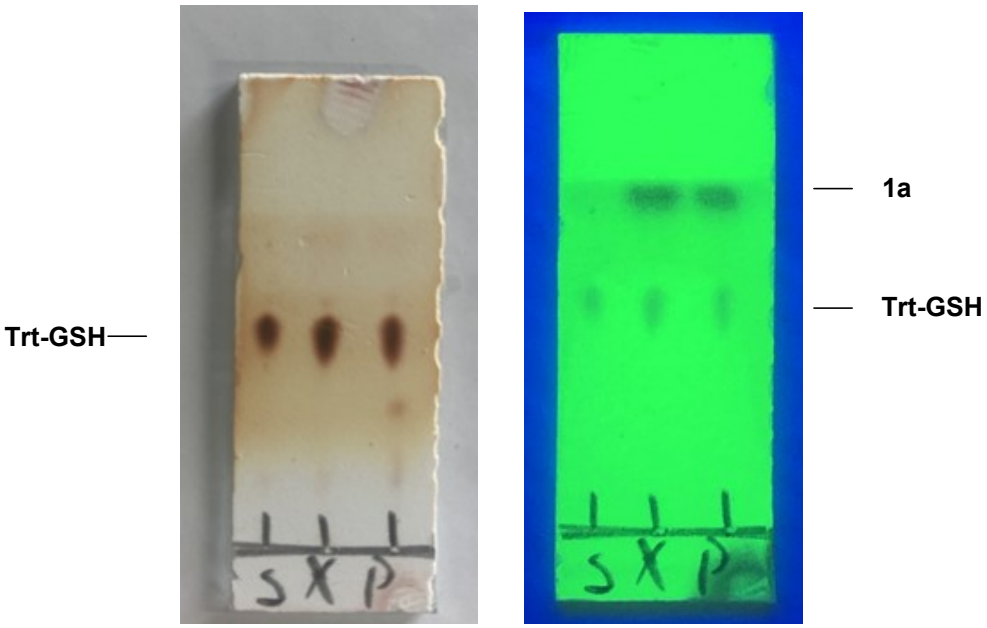
Trt-GSH

Trt-GSH: The experimental step refers to Kohsaku's method.[7] The product **Trt-GSH** was afforded as a white solid (1.5 g, 88%).[1]H NMR (300 MHz, Methanol-d4) δ 7.31 (d, J = 7.8 Hz, 5H), 7.26 – 7.06 (m, 10H), 4.23 – 4.14 (m, 1H), 3.84 (t, J = 6.3 Hz, 1H), 3.77 (d, J = 4.3 Hz, 2H), 2.53 (td, J = 12.6, 5.6 Hz, 2H), 2.42 (t, J = 7.1 Hz, 2H), 2.13 – 1.99 (m, 2H).The data are consistent with those reported in the literature.[7]

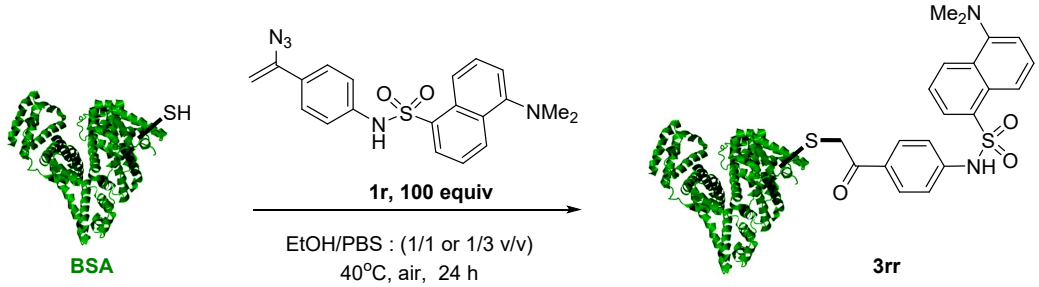


Trt-GSH **1a**

The reaction of Trt-GSH with **1a** was carried under standard conditions, even stirred in air for 24 hours, no reaction was observed by TLC.



6. Labeling of BSA with dansyl fluorescent probe 1r



To a 1.5 mL centrifuge tube, was added 10 μL BSA (0.1 g/ml, 1 mg, 1.5×10^{-6} mmol, 1 equiv, prepared from 0.1g/mL BAS of PBS solution), followed by 40 μL EtOH and 120 μL PBS (10 mmol/L, pH 7.4) or 90 μL EtOH and 90 μL PBS. To the premixed solution, was added 10 μL **1r** (0.06 mg, 1.5×10^{-4} mmol, 100 equiv, prepared from 6 mg/mL **1r** in EtOH). The resulting mixture (final volume: 200 μL, [protein] = 1.5×10^{-6} mmol) was covered with plastic wrap and punched, then it was gently shaken at 40 °C for 24 h. After the reaction, the samples were analyzed by SDS-PAGE.

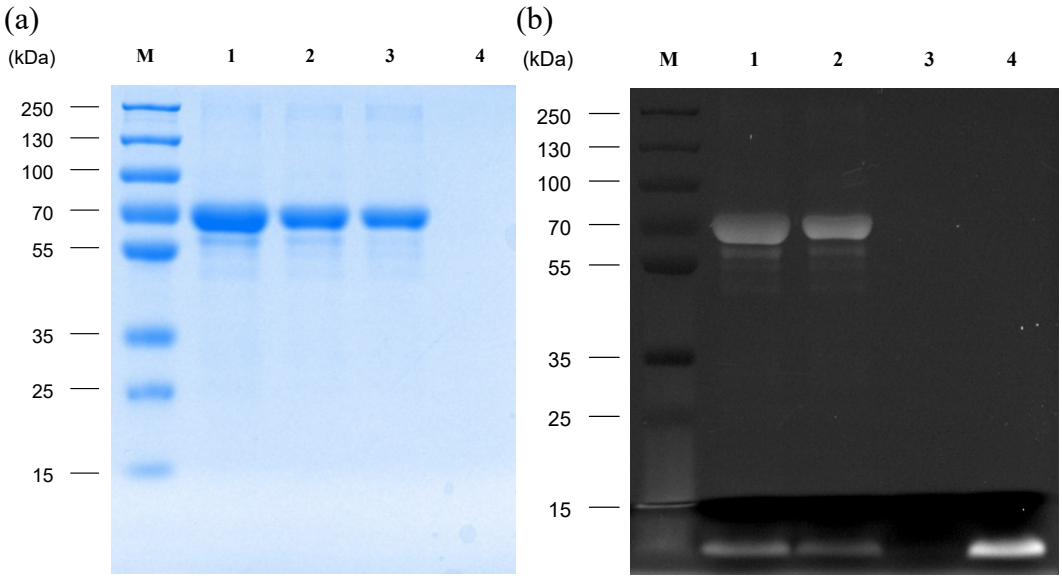


Figure S13. SDS-PAGE analysis of BSA modified with dansyl-vinyl azides. Lane 1: Reaction in 1/1 EtOH/PBS (v/v), BSA dansyl conjugate **3rr** was treated with 5×SDS-PAGE sample buffer and boiled at 100 °C for 5 min; Lane 2: Reaction in 1/3 EtOH/PBS (v/v), BSA dansyl conjugate **3rr** was treated with 5×SDS-PAGE sample buffer and boiled at 100 °C for 5 min; Lane 3: unmodified BSA; Lane 4: dansyl-vinyl azides **1r**. (a) CBB stain of the gel. (b) Fluorescence image of the gel. **M**: PageRuler Plus Prestained Protein Marker.

7. Labeling of Ni-NTA resin with YPet-ECFP and STAV AF568

The ^{Biotin}**1s** was dissolved in THF and diluted to 8.4×10^{-3} μmol/μL; Streptavidin Alexa Fluor 568 conjugate (American Thermo company) was dissolved in PBS (pH 7.4) and diluted to 36 μM. The purified **YPet-ECFP**'s protein concentration was 0.5808 μg/μL, which was determined by BCA Protein Assay Kit (Sigma-Aldrich, Germany). The reaction was conducted in two 500 μL centrifuge tubes **(1)** and **(2)**. To tube number **(1)**, were added ^{Biotin}**1s** (10 μL, 8.4×10^{-2} μmol, 100 equiv) and **YPet-ECFP** (100 μL, 8.4×10^{-4} μmol, 1 equiv). To tube number **(2),** was added **YPet-ECFP** (100 μL, 8.4×10^{-4} μmol, 1 equiv). Both of two reaction mixtures were gently shaken at 37 °C for about 40 hours. To the two reaction mixtures, were respectively added 50 μL Nickel NTA agarose beads (Ni-NTA) (purchased from Thermo Scientific) and shaken for 1 h at room temperature to ensure that **YPet-ECFP** was fully bound with the Ni-NTA Resin. The Ni-NTA resin and it's adsorbates were precipitated by centrifugation in a low-speed centrifuge, and the supernatant was removed. Each tube was washed three times with PBS to wash off excess ^{Biotin}**1s**. Streptavidin Alexa Fluor 568 conjugate (12.5 μL, 4.5×10^{-4} μmol, 36 μM) and PBS were respectively added, which were later shaken at room temperature for 5 min. Two reaction mixtures were washed three times with PBS to remove excess Streptavidin Alexa Fluor 568 conjugate. Finally, 100 μL PBS was added to resuspend the Ni-NTA resin and two mixtures were placed on the bottom of the confocal dish for microscope imaging (Carl Zeiss, Germany, primo vert).

8. References

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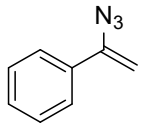
9. NMR spectra of compounds



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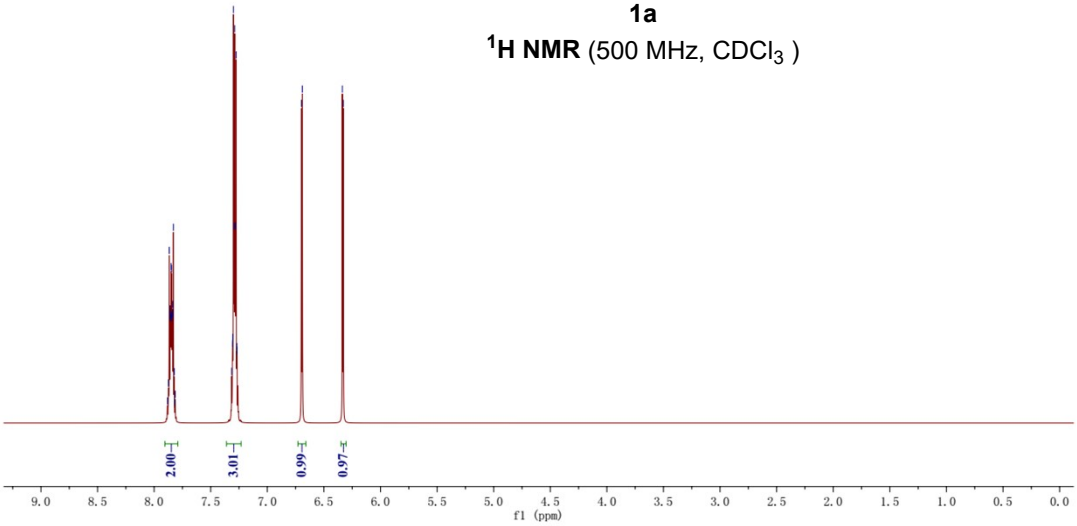
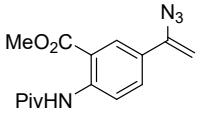
^{1}H NMR (500 MHz, $CDCl_3$)





1b

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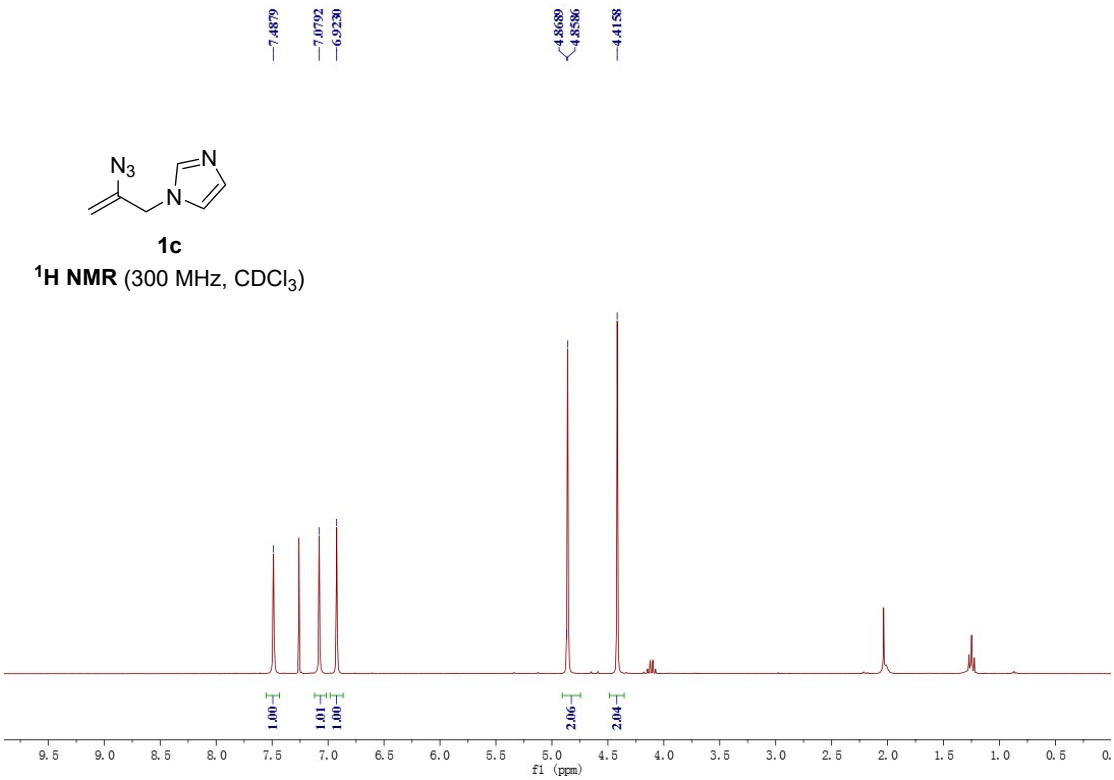

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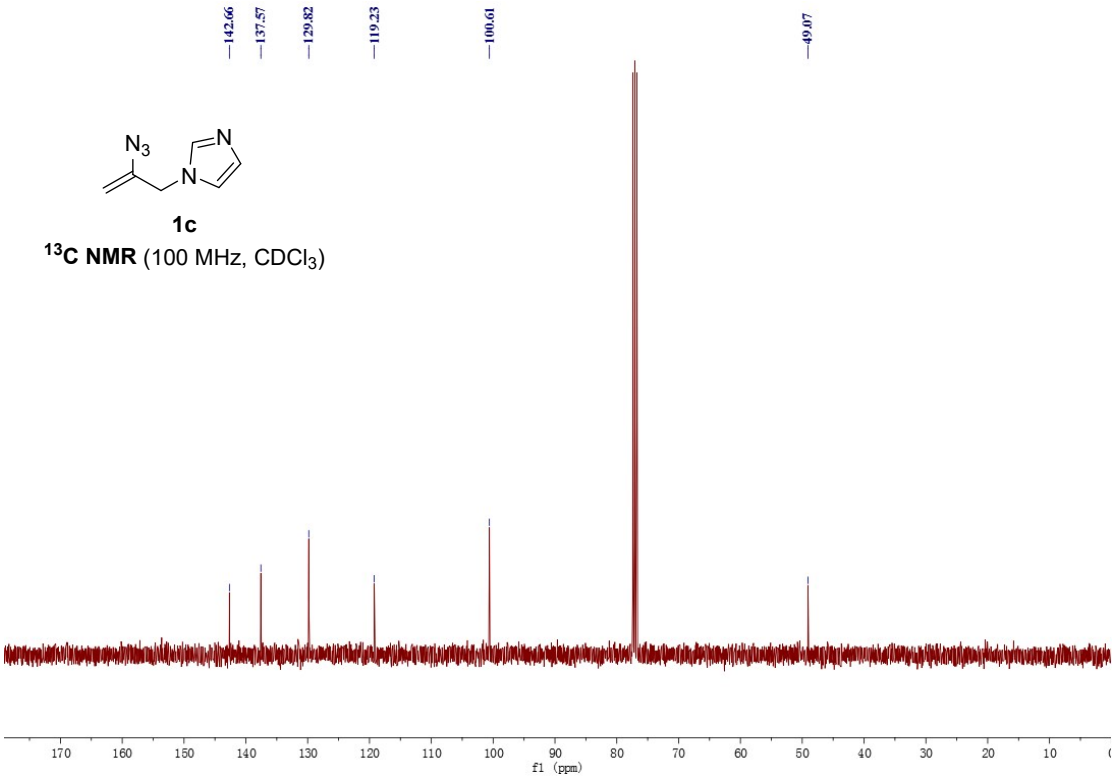

1c

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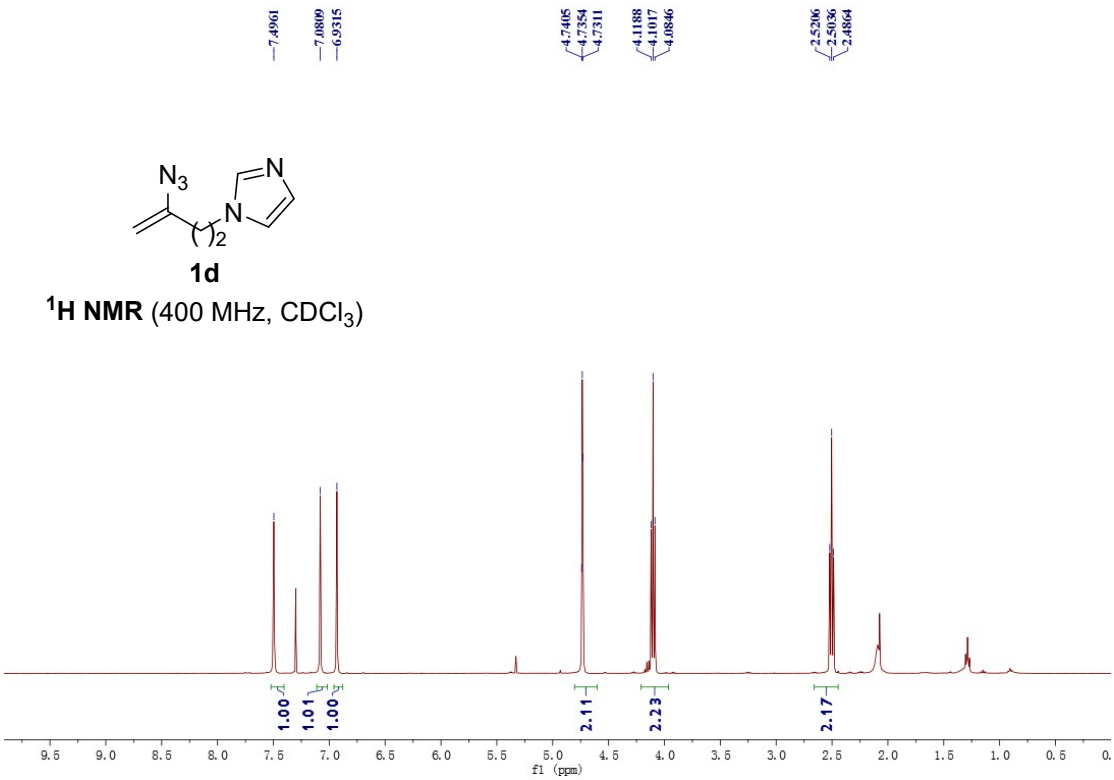

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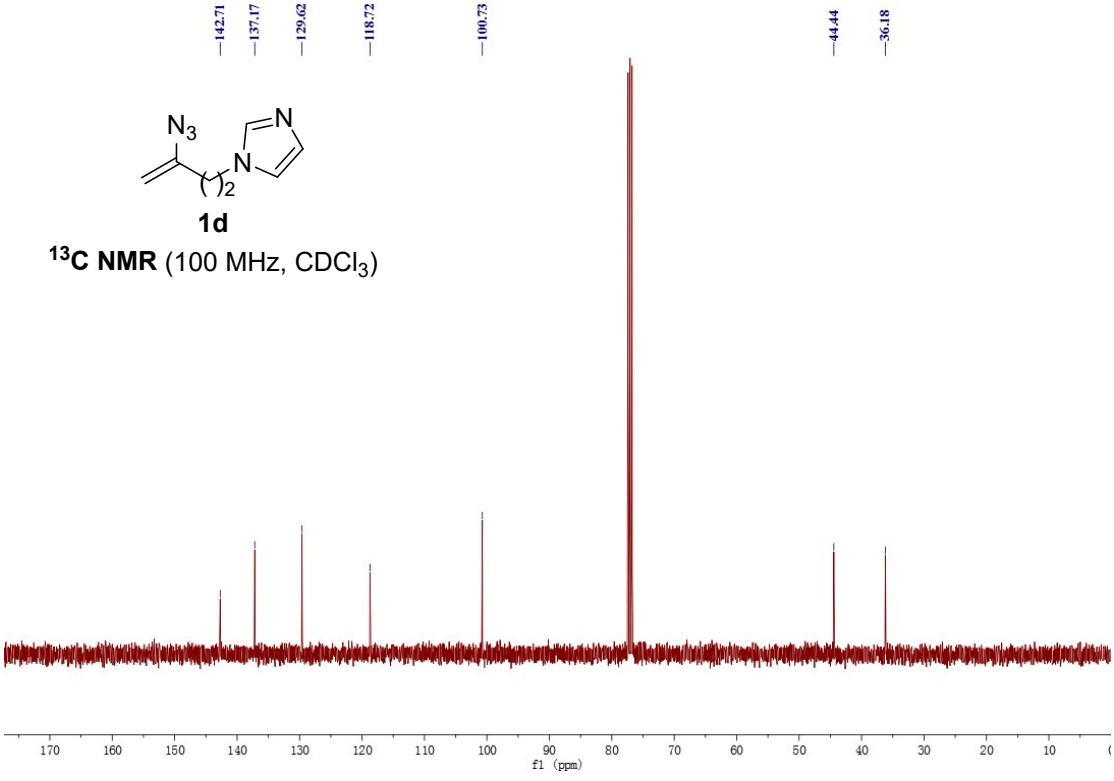

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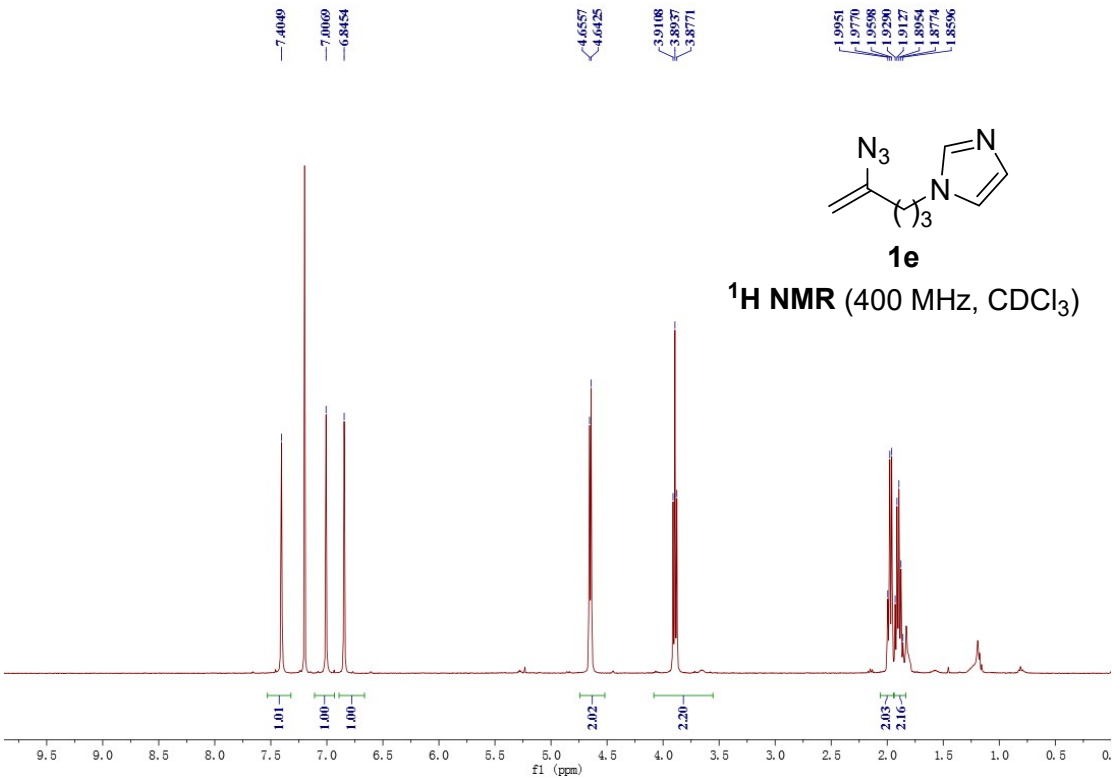

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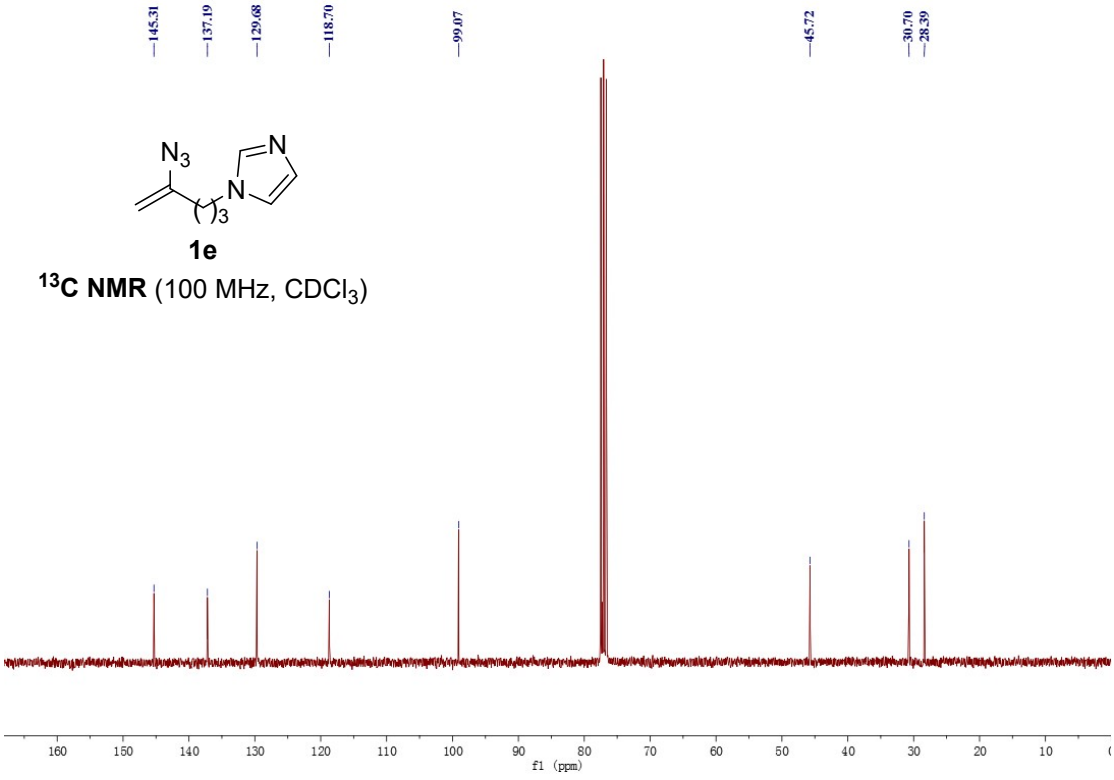

1e

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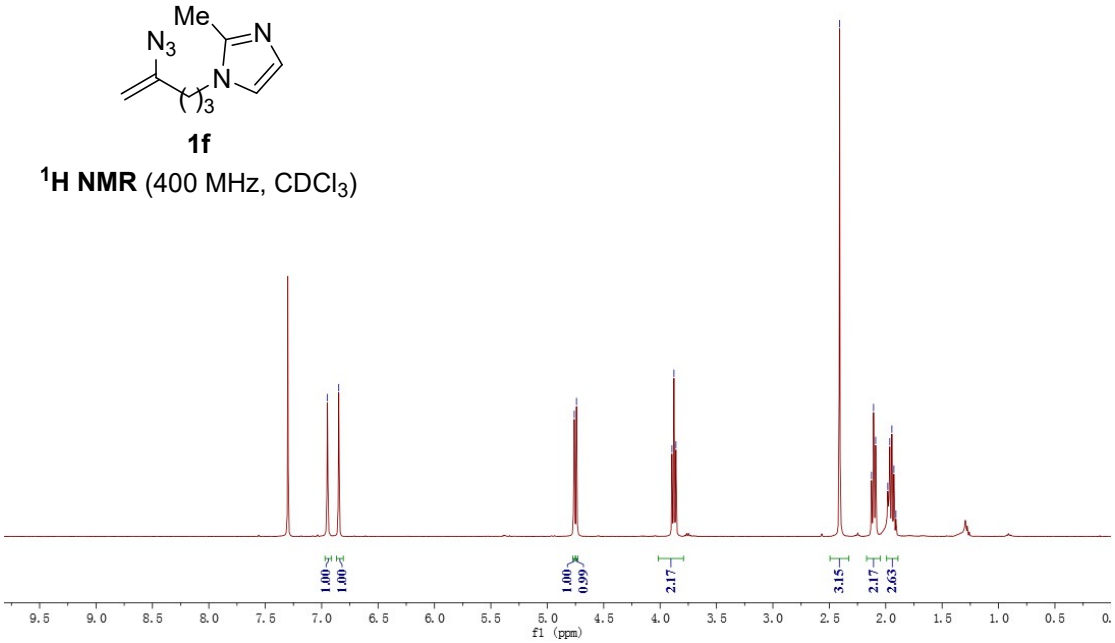
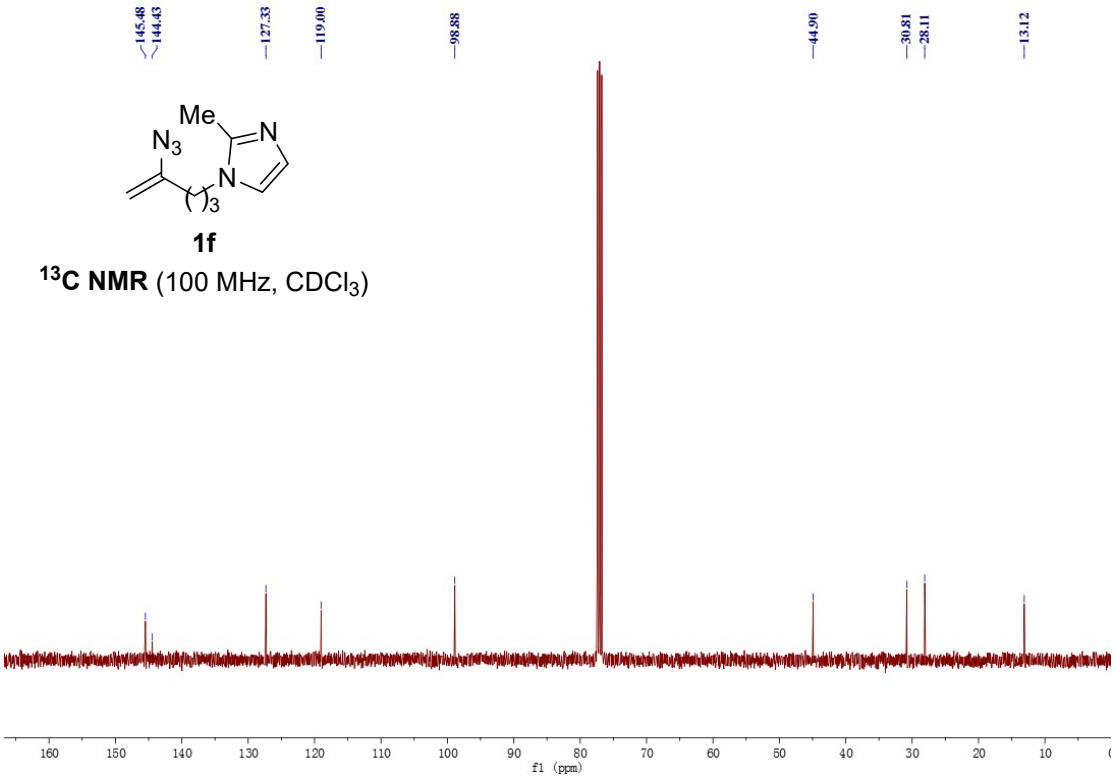


1f

^{1}H NMR (400 MHz, $CDCl_3$)



1f

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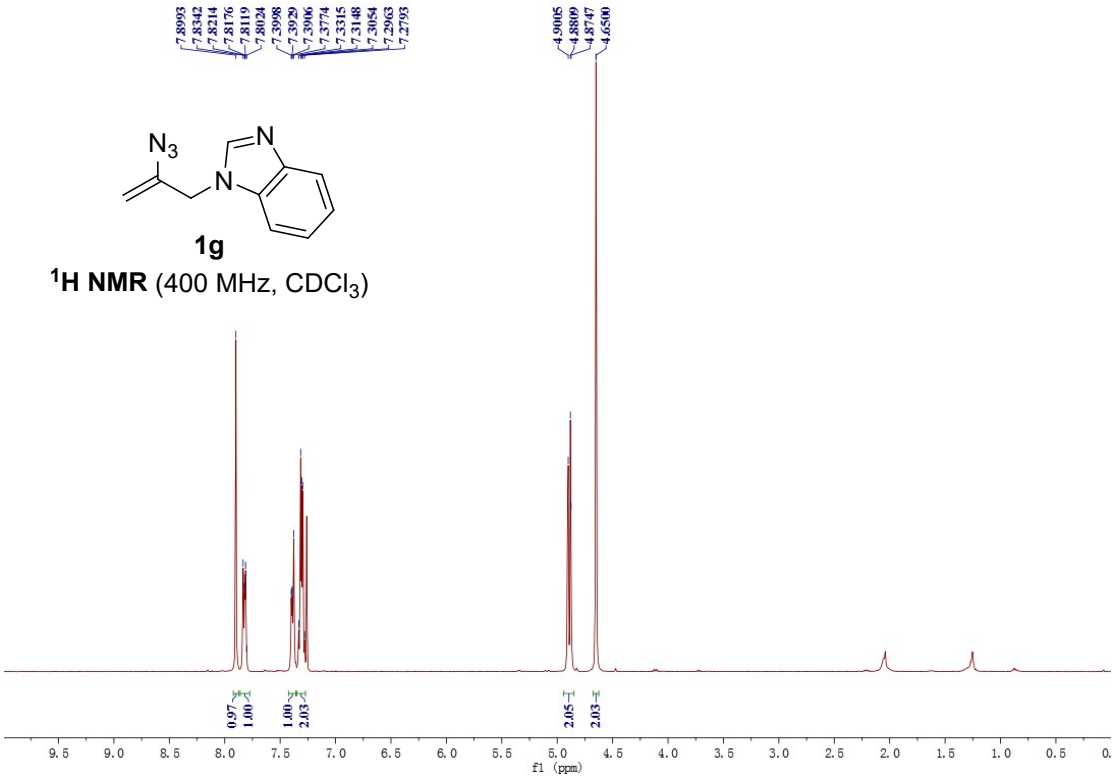

1g

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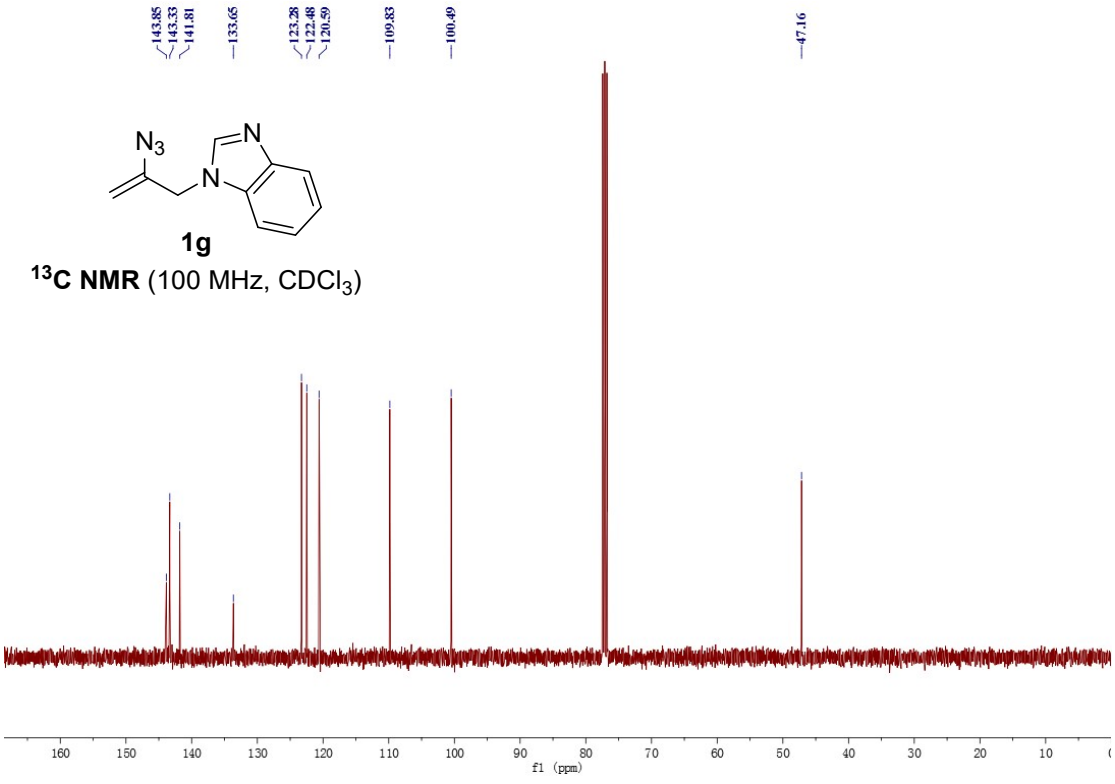

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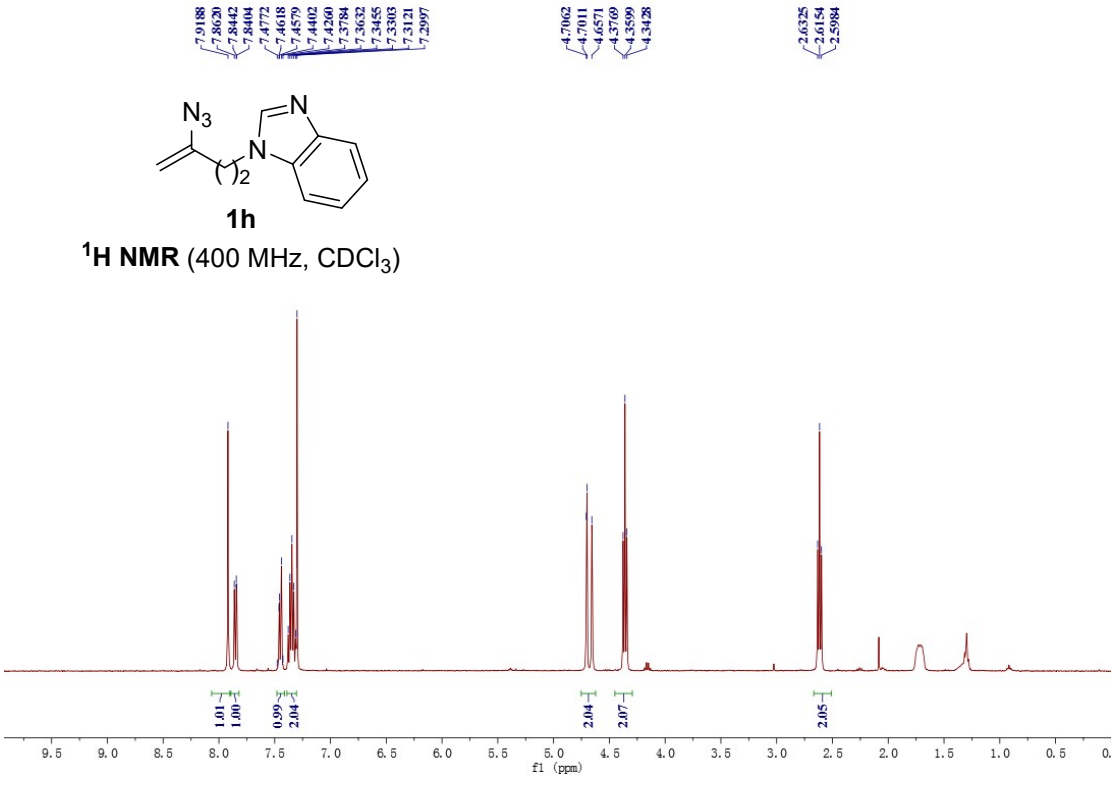

1h

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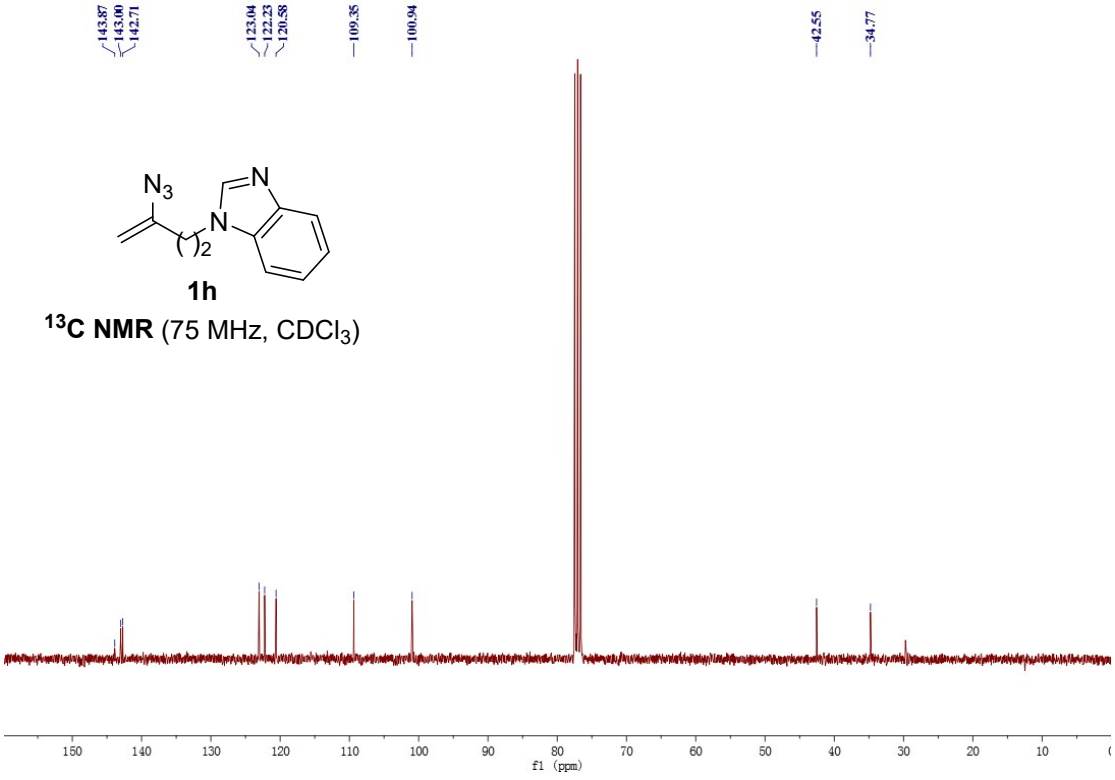

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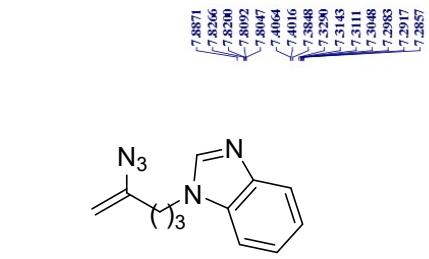

1i

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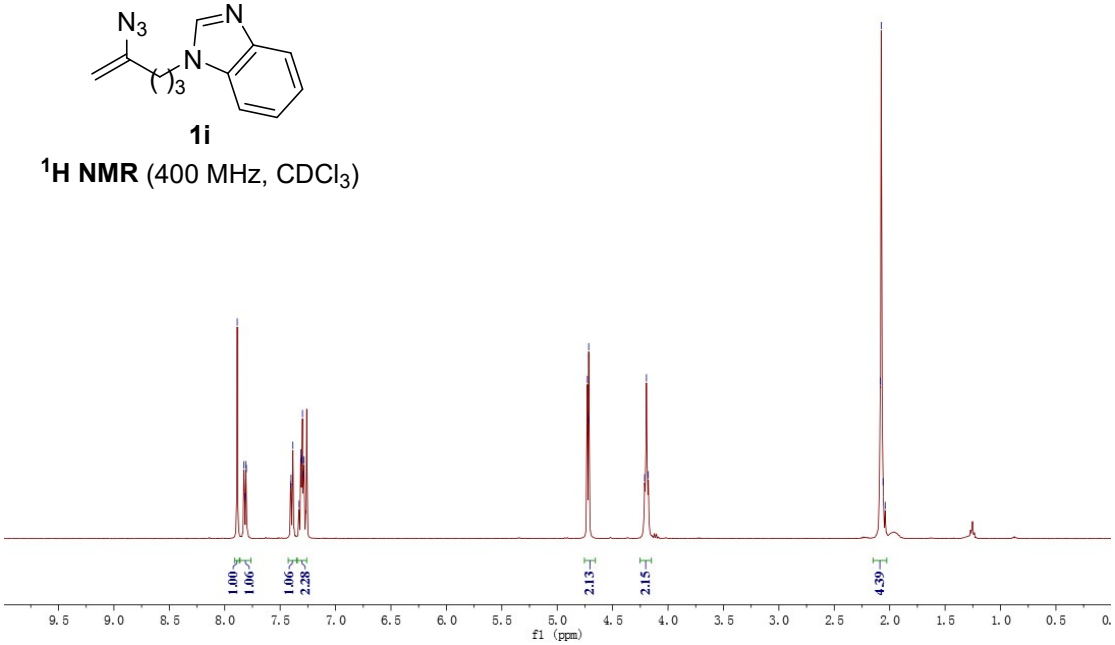

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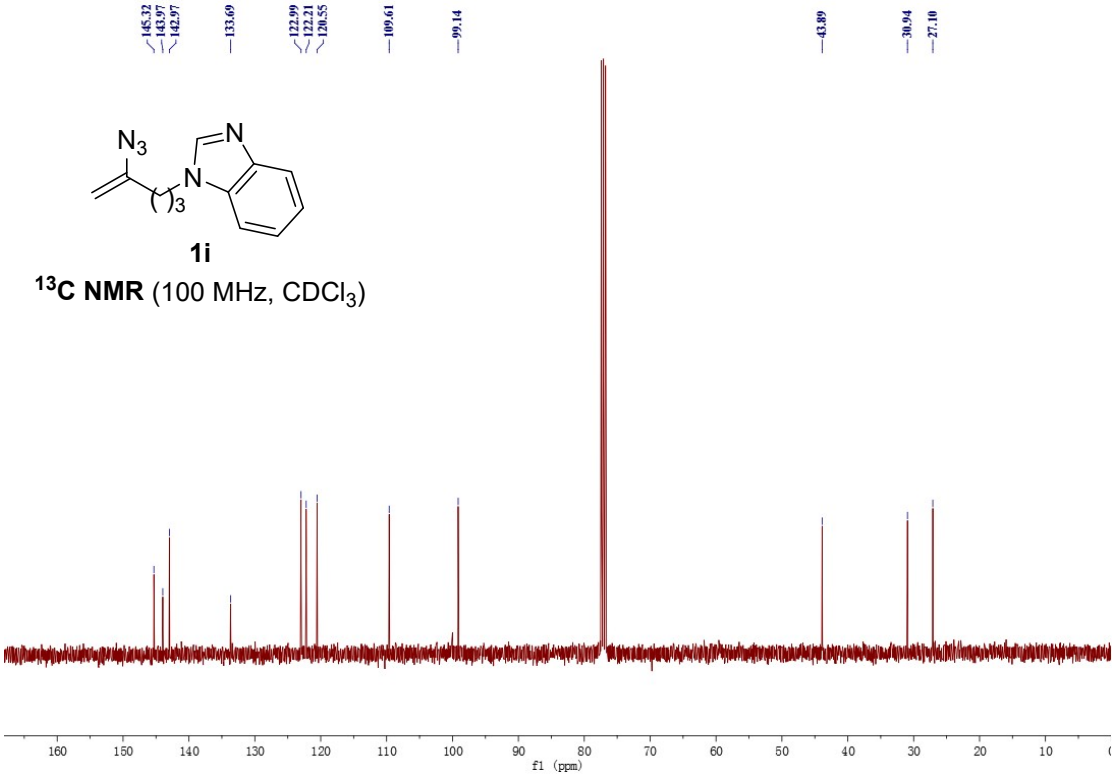

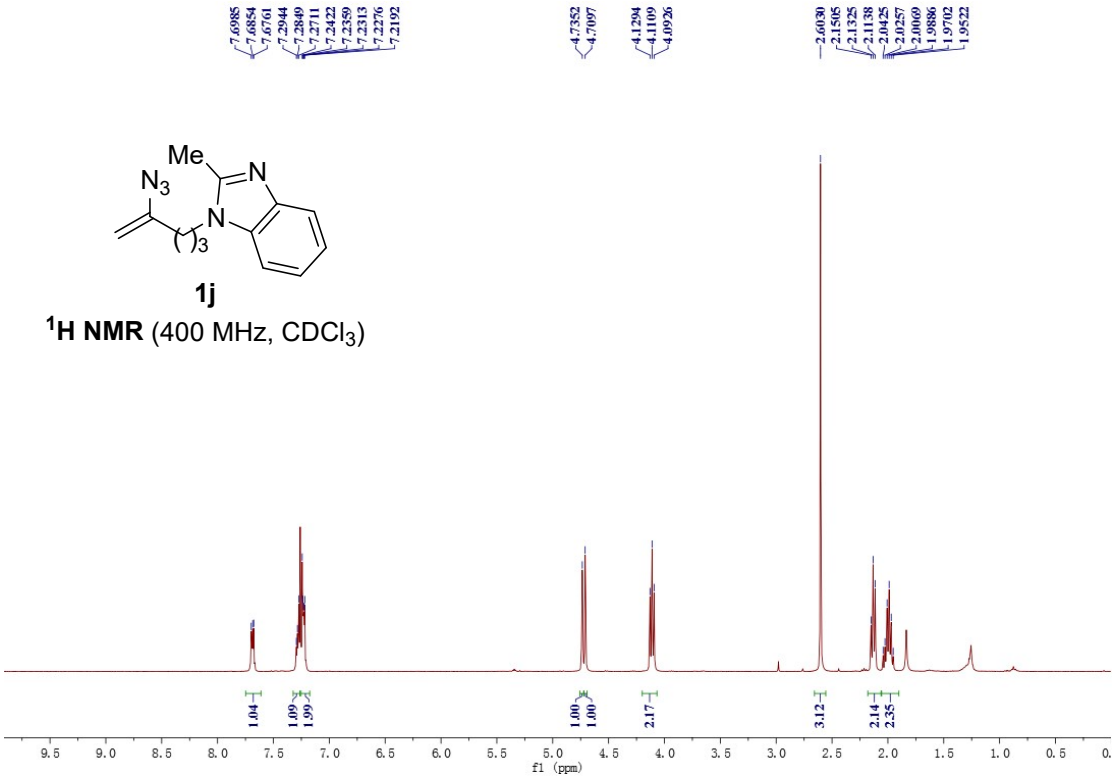

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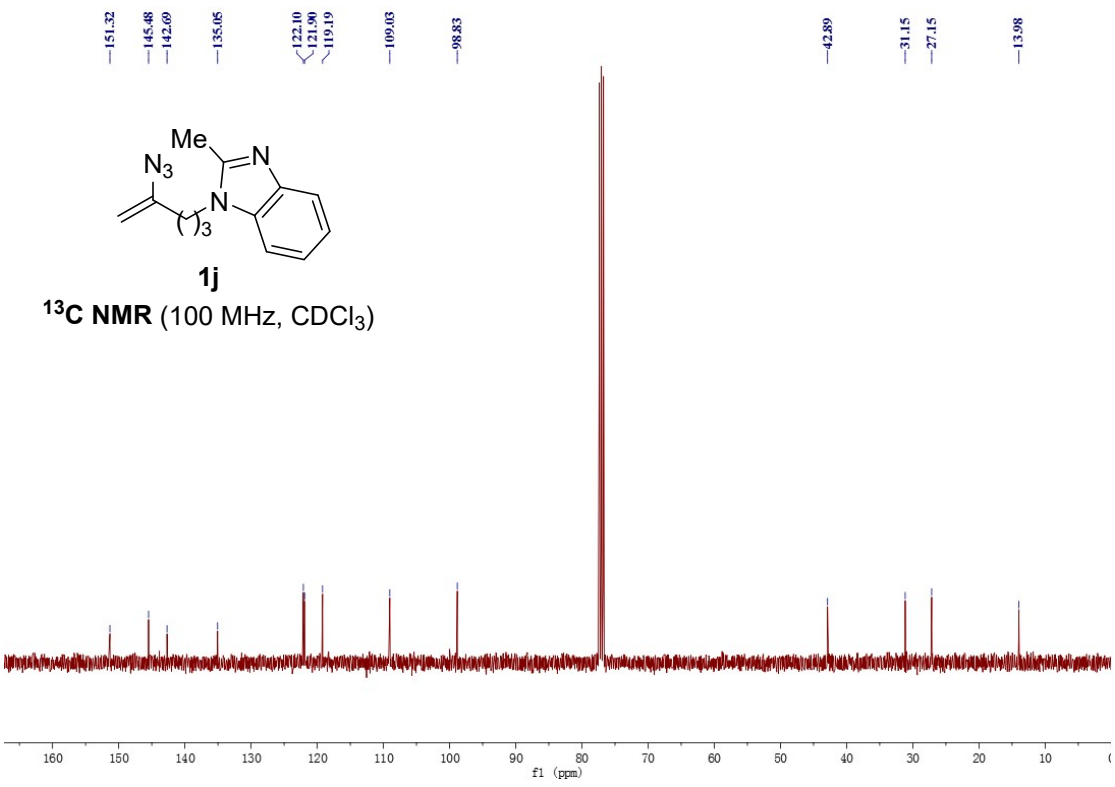

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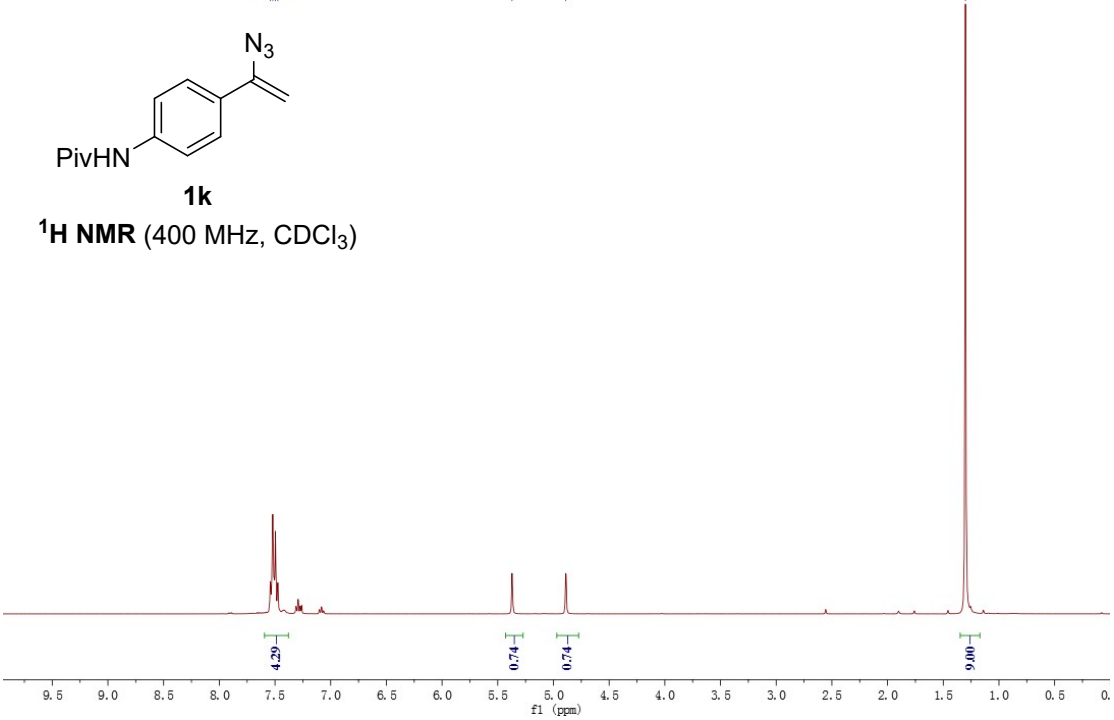

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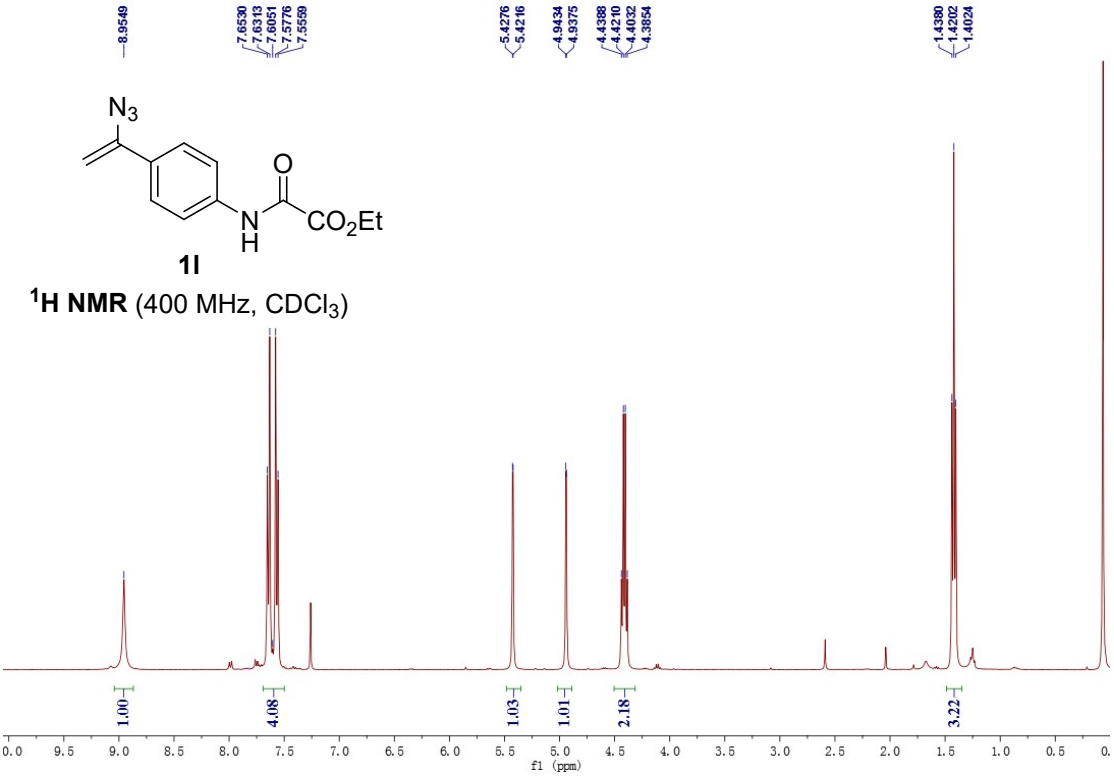

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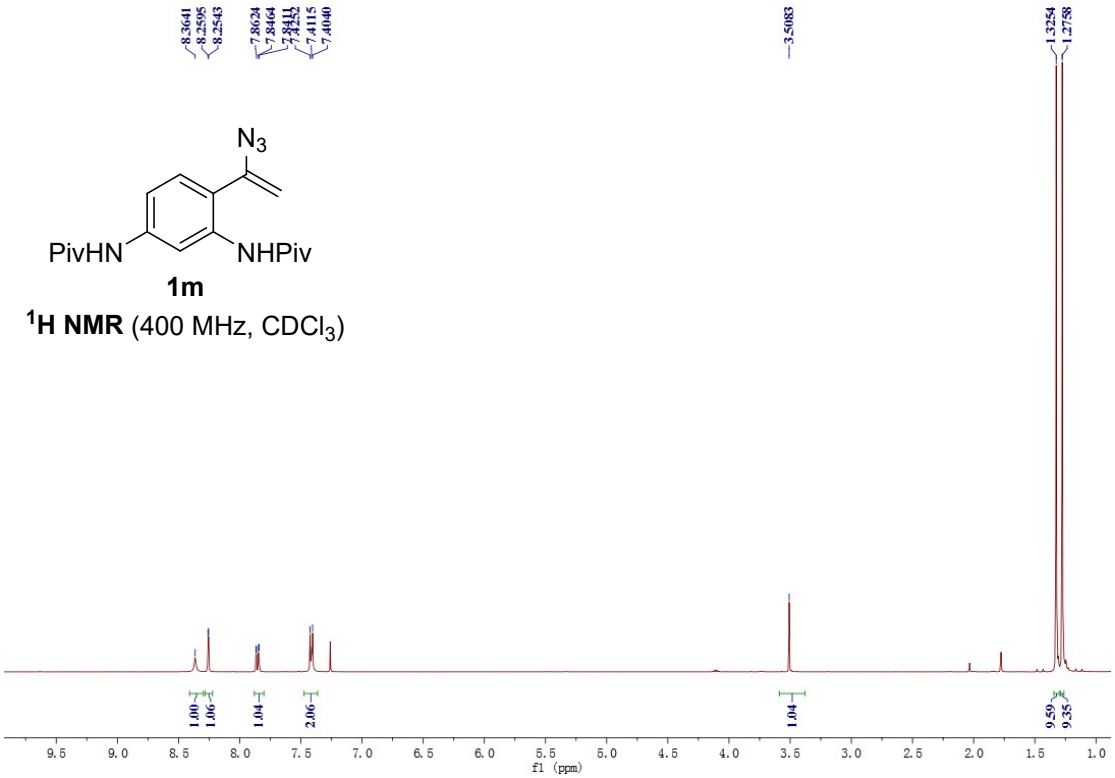

1m

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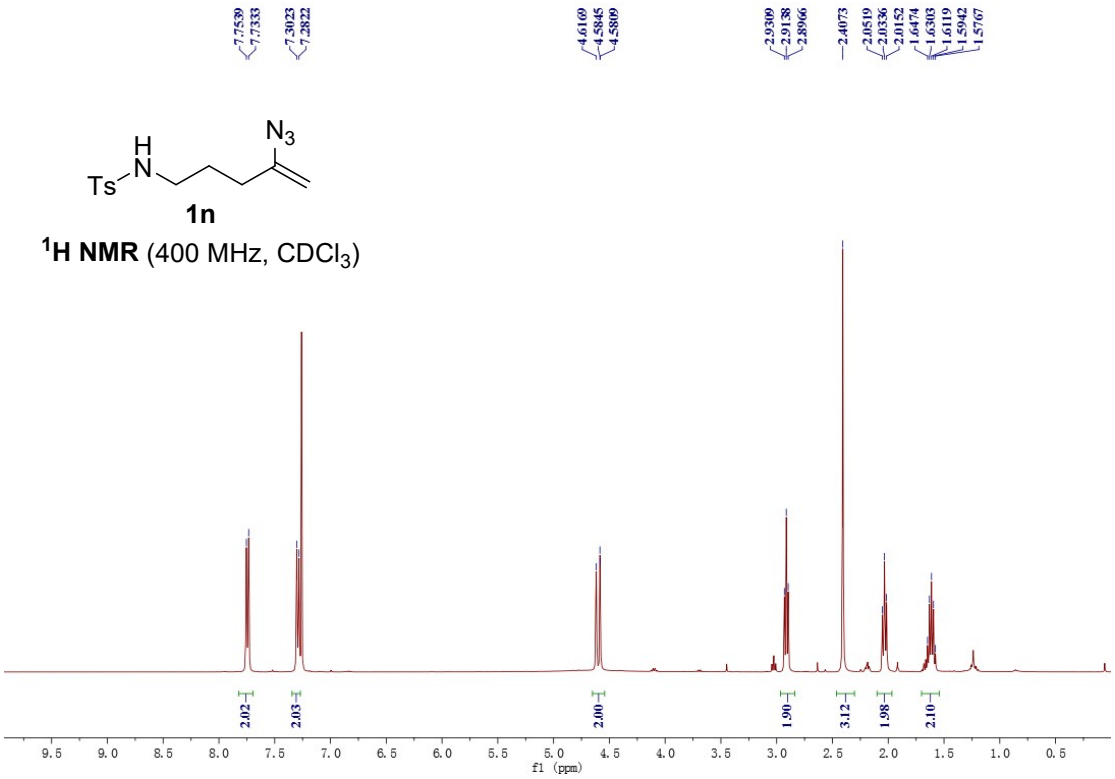

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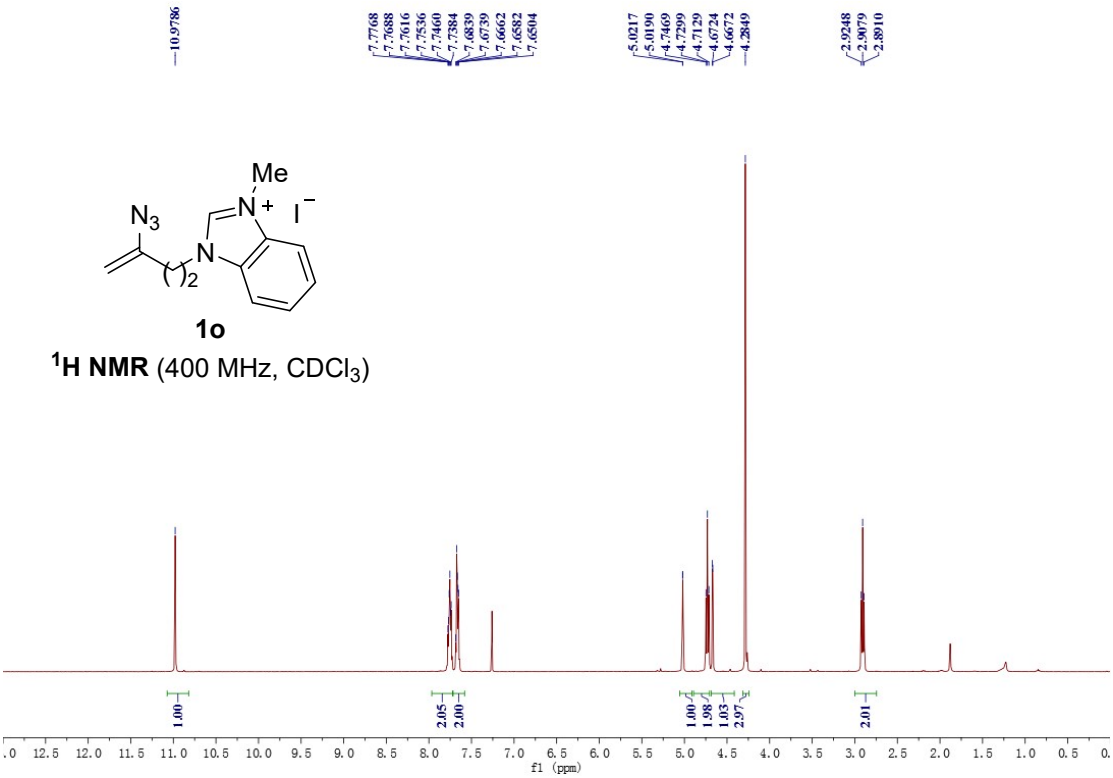

1o

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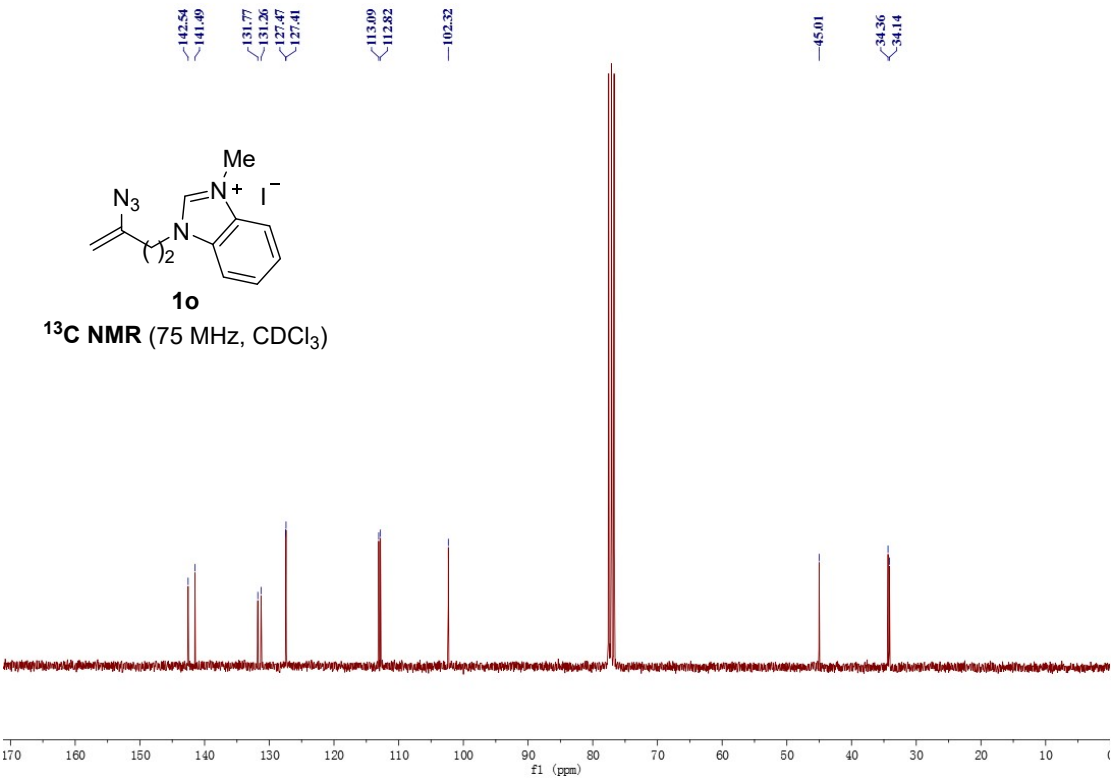

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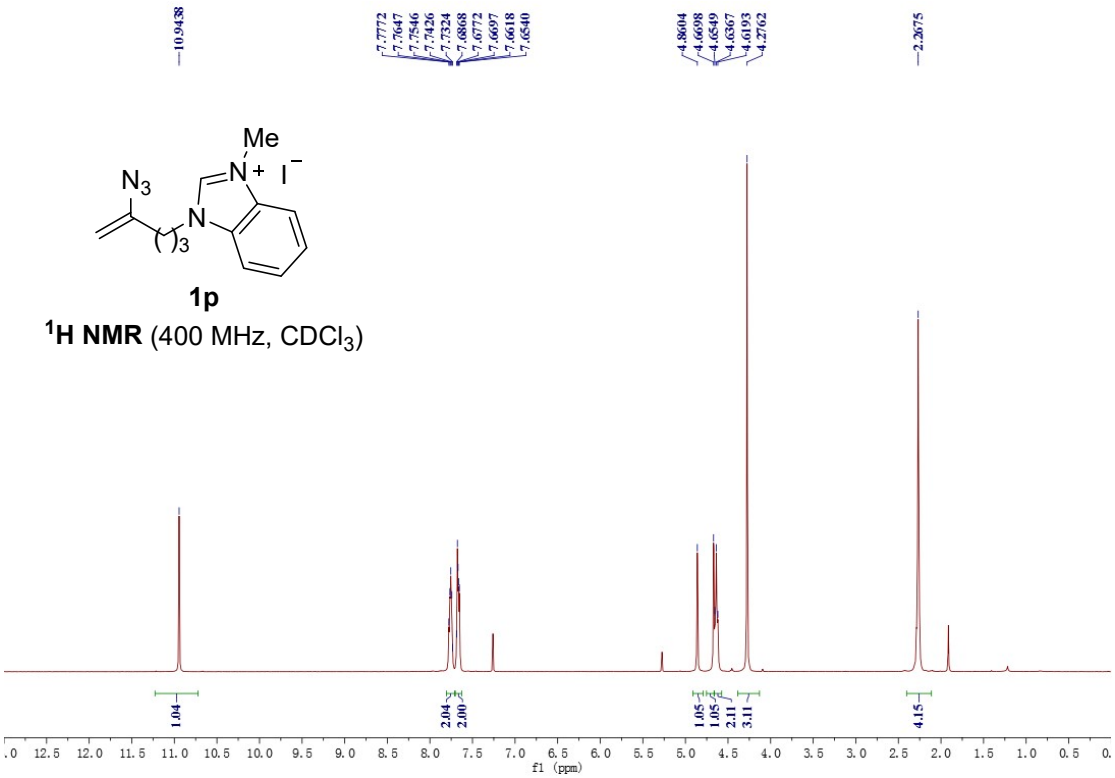

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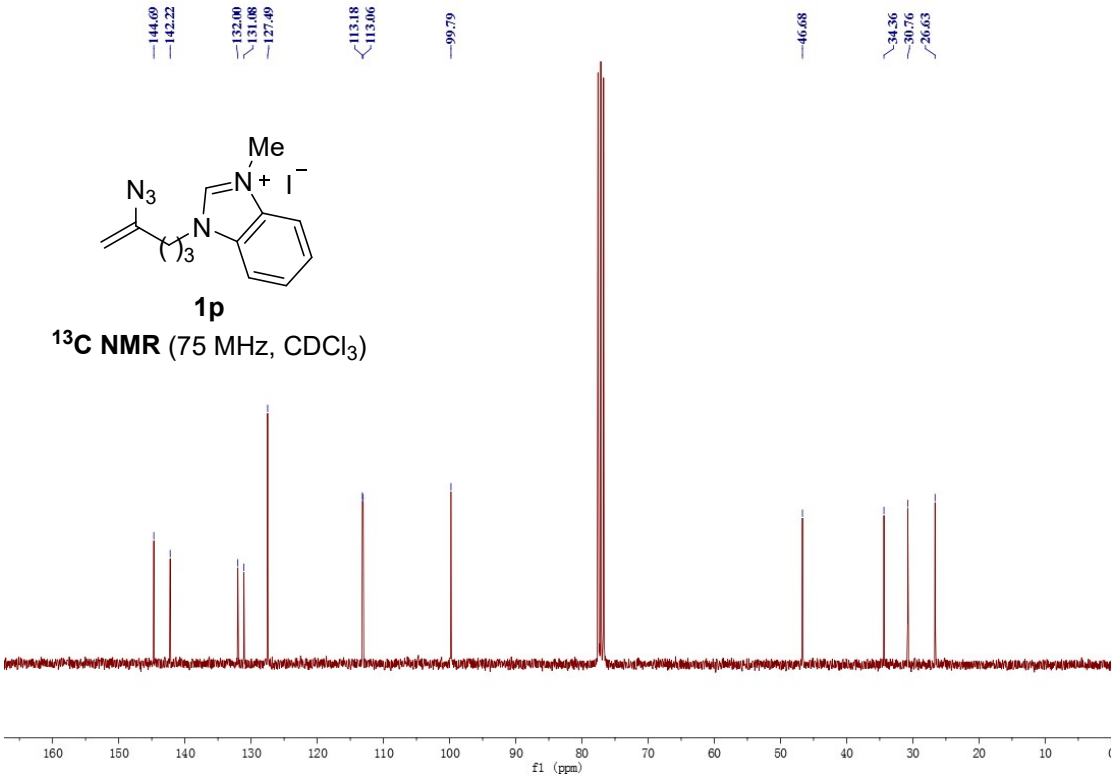

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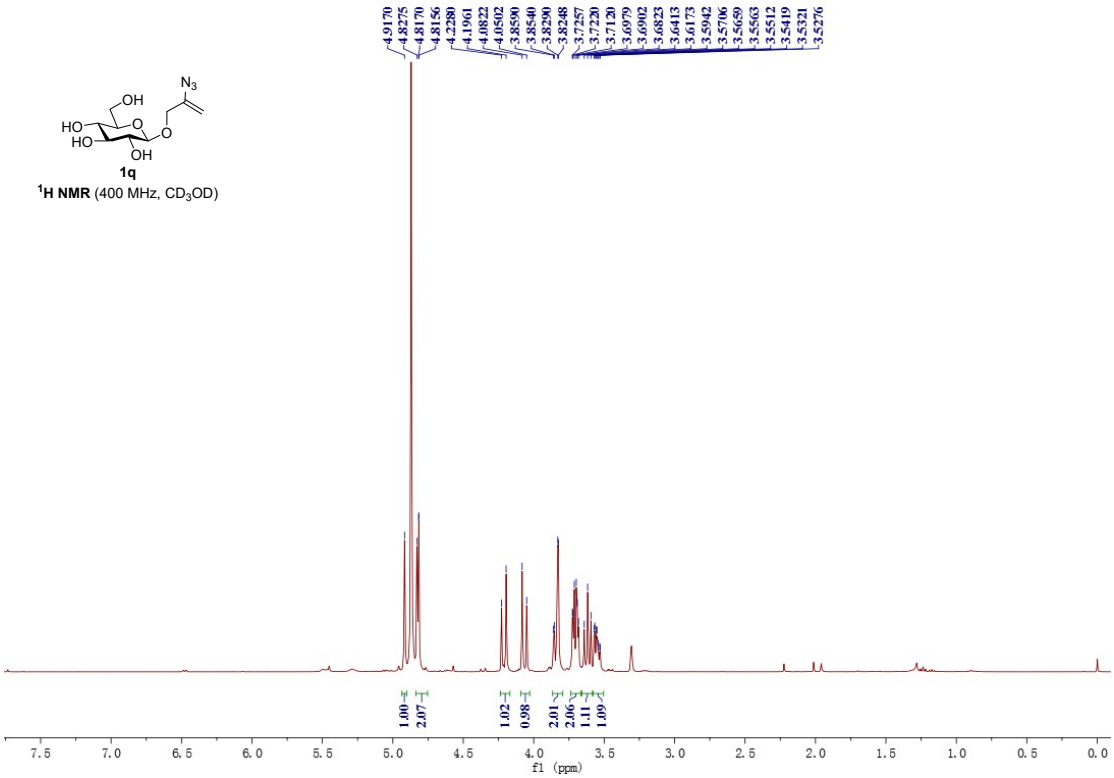

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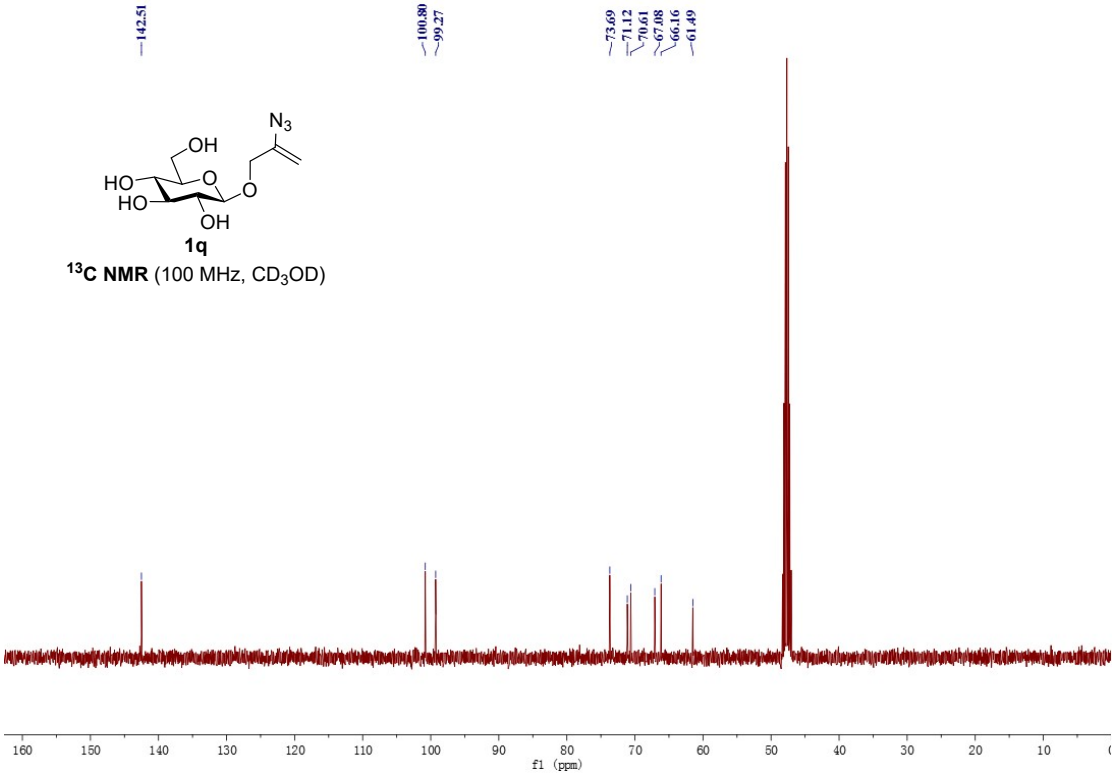

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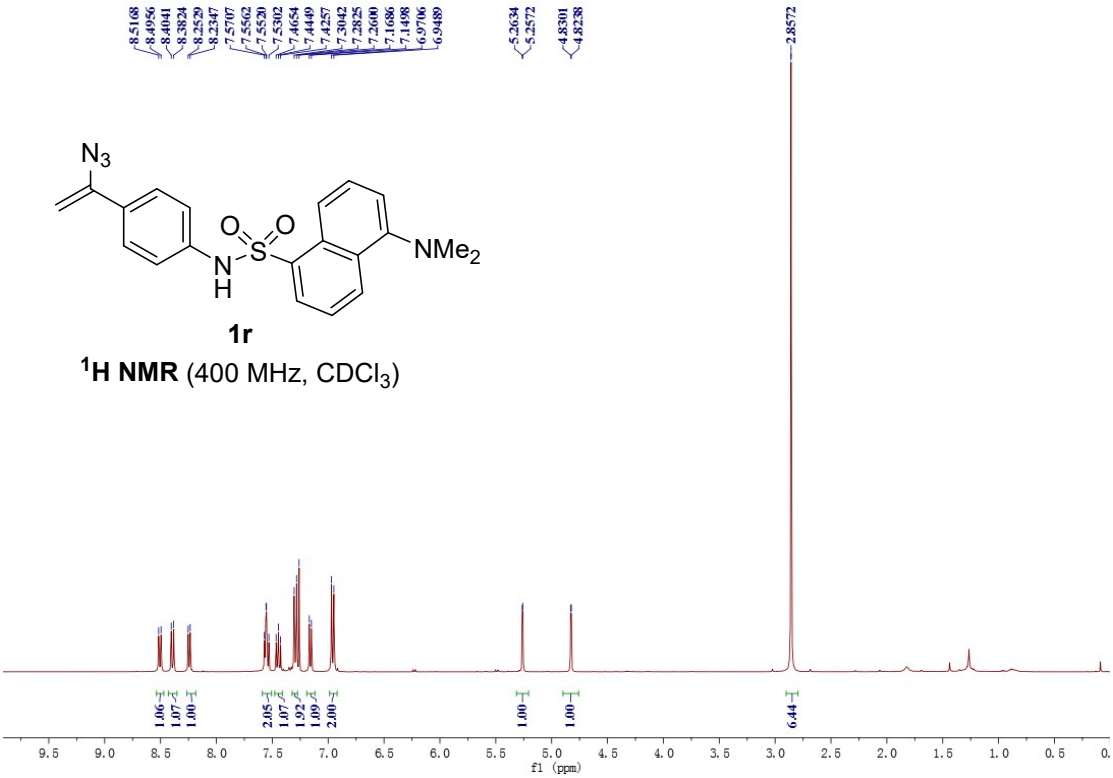

1r

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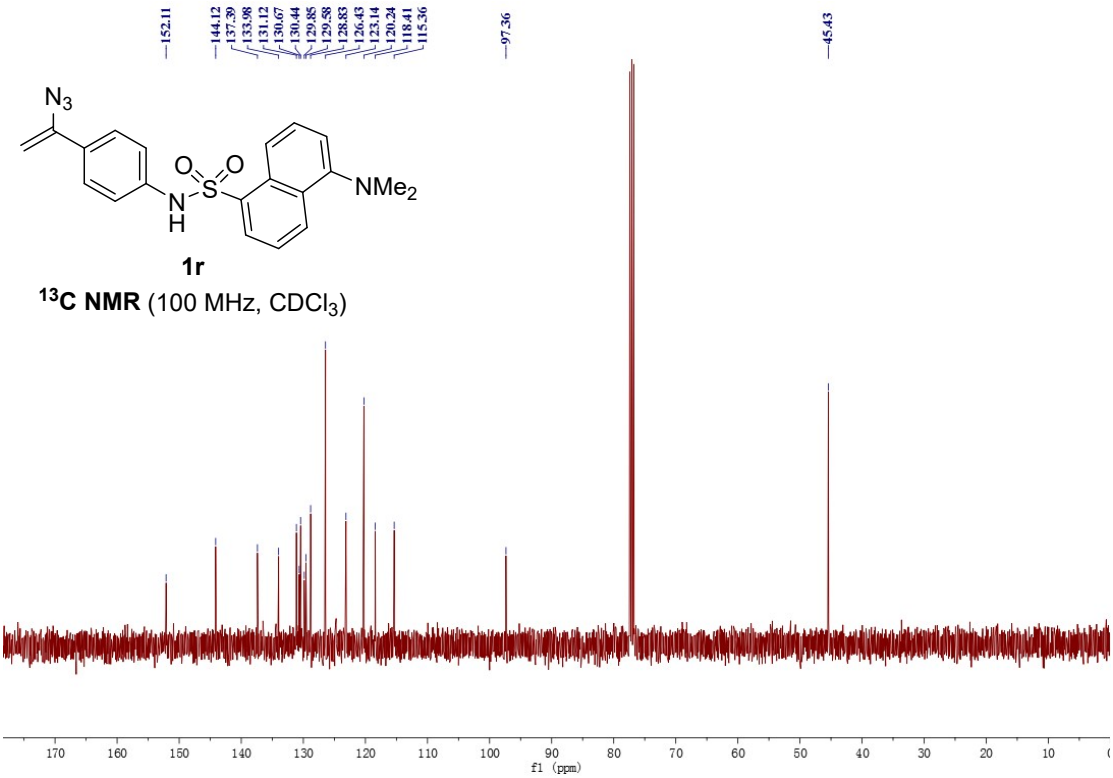

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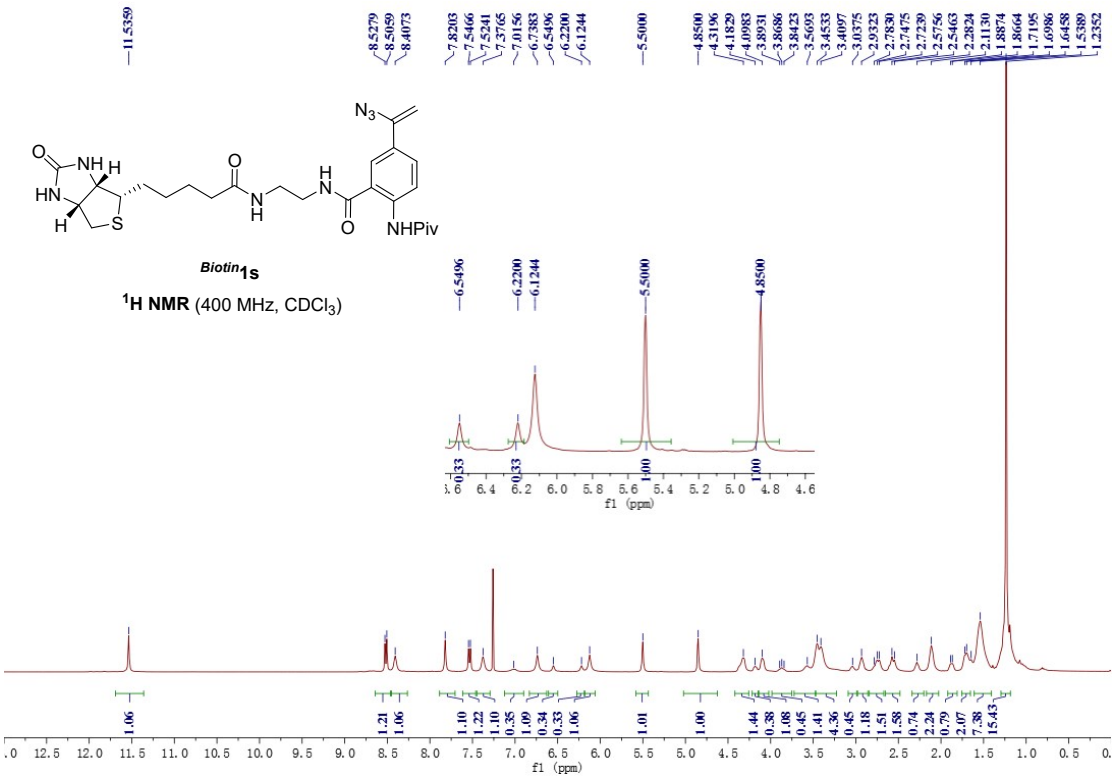

***Biotin*1s**

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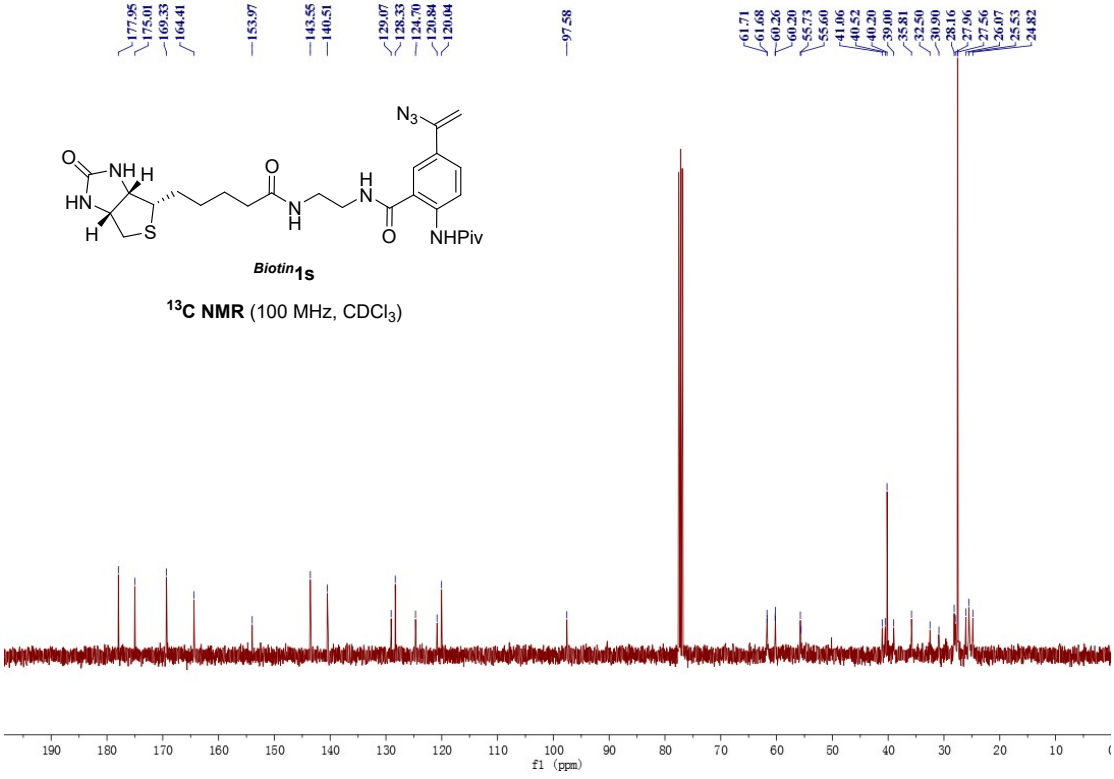

***Biotin*1s**

^{13}C NMR (100 MHz, $CDCl_3$)

2a

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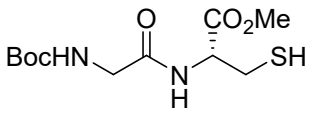

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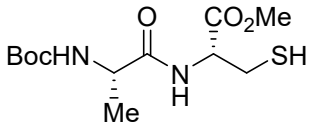

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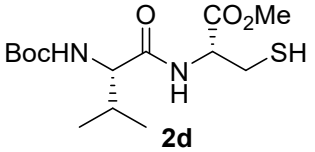

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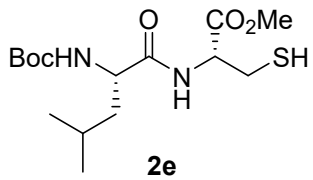

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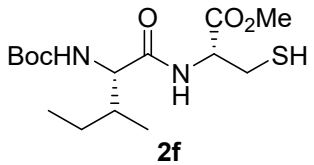

2f

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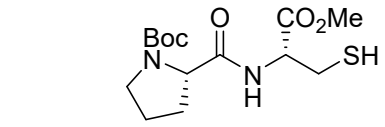

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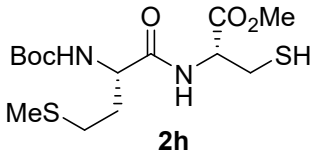

2h

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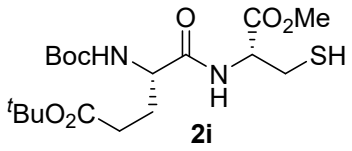

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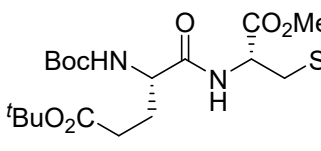

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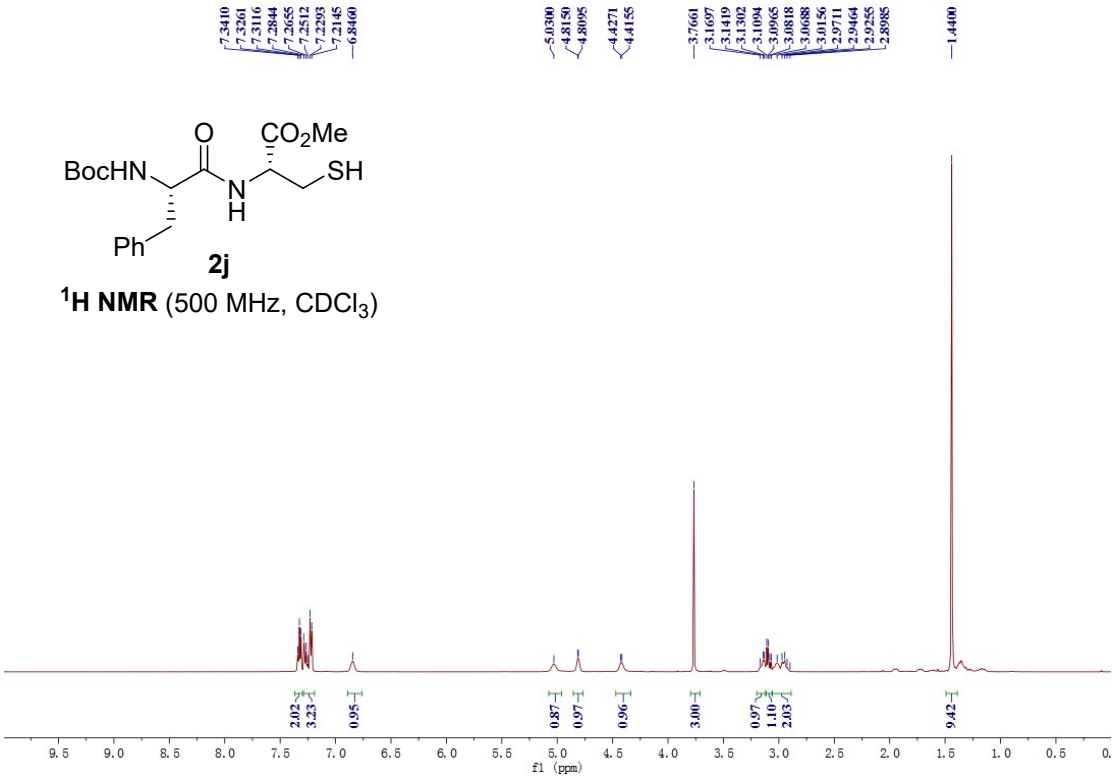

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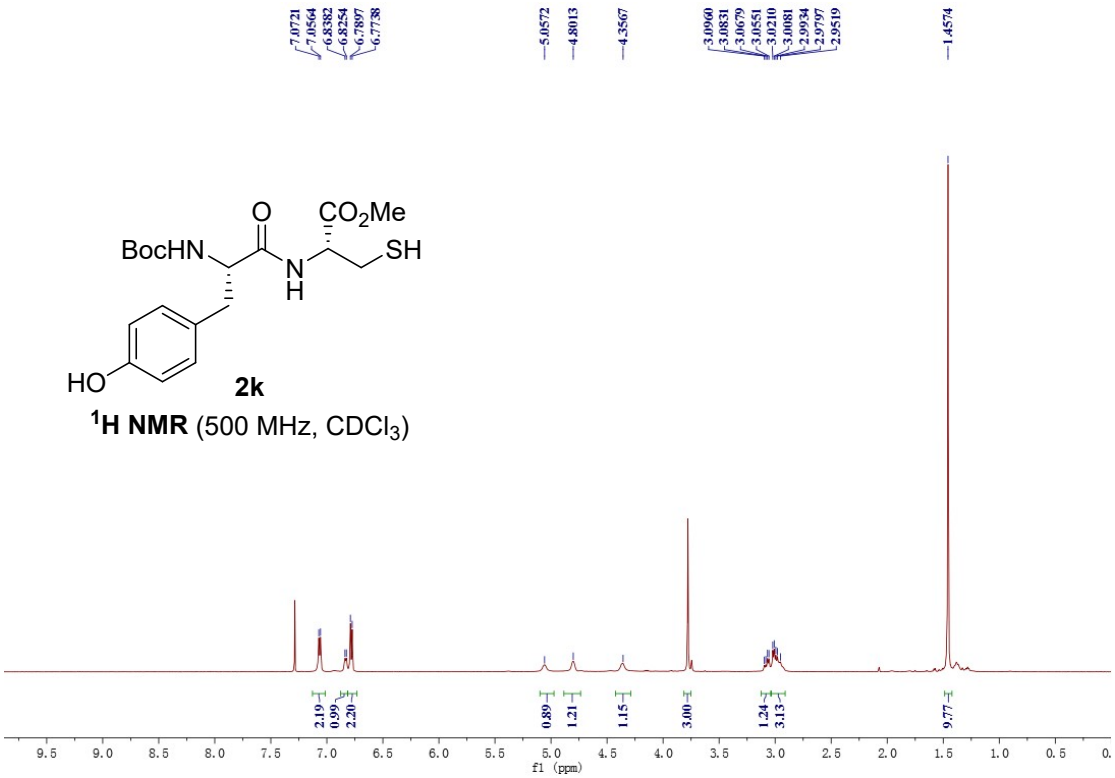
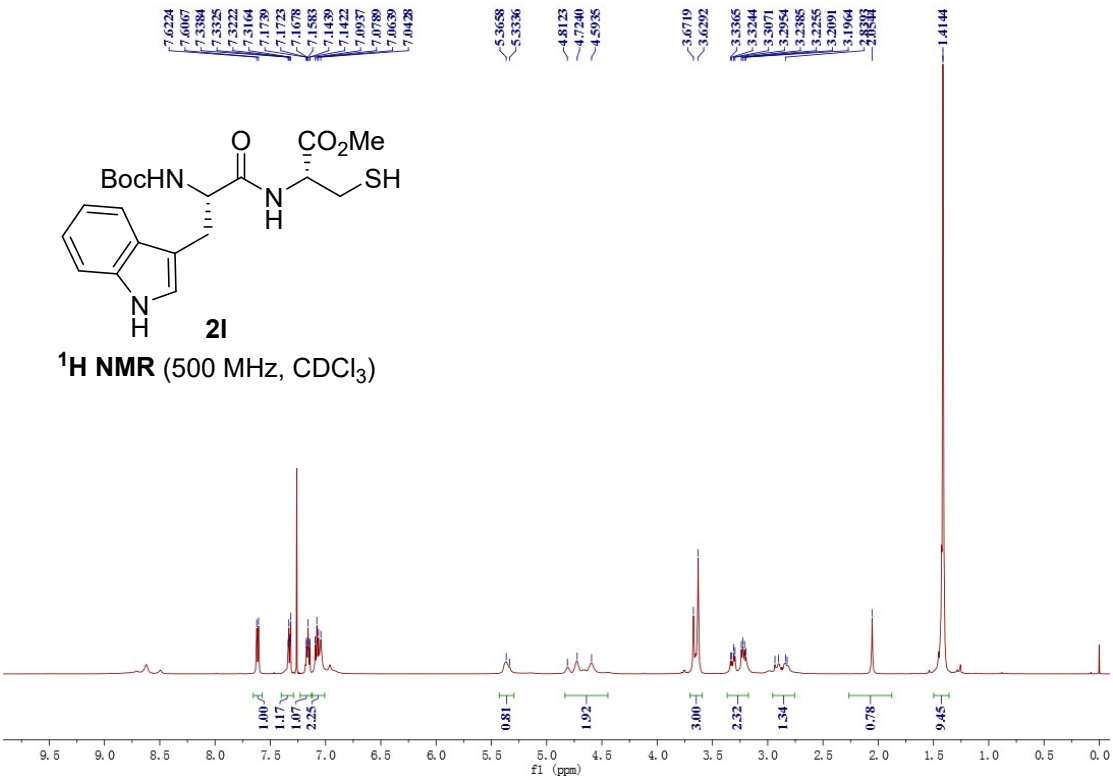


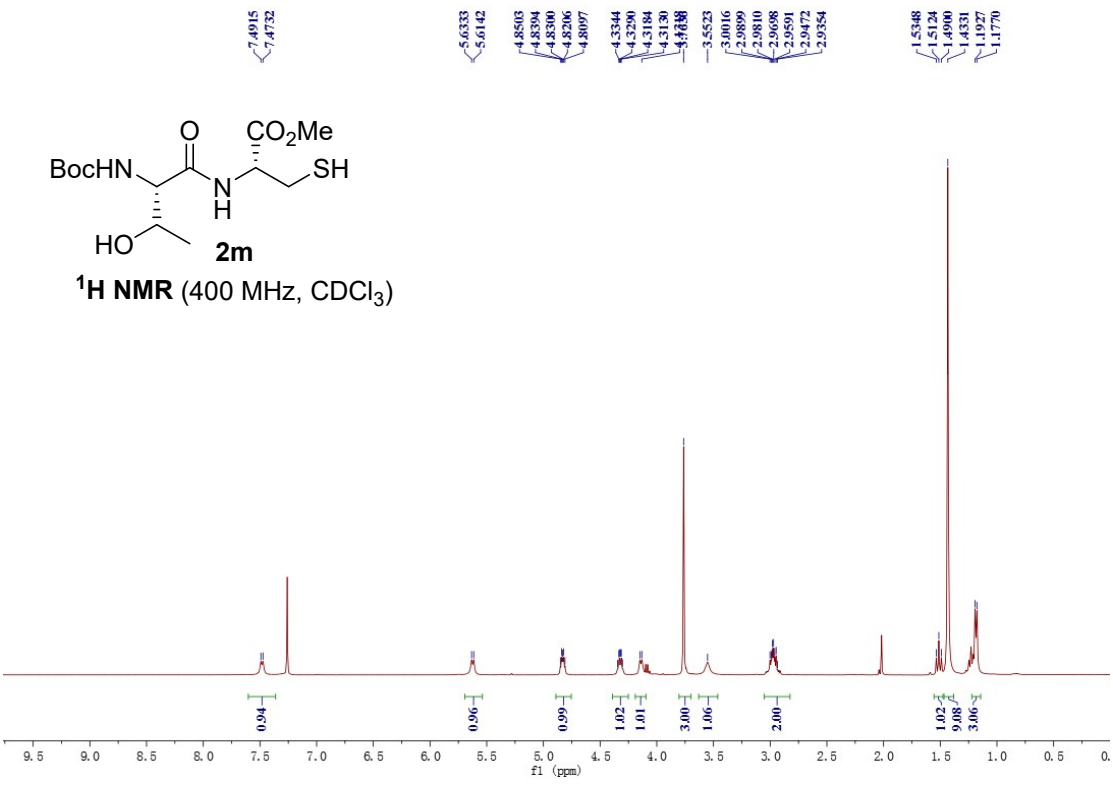
2k

^{1}H NMR (500 MHz, $CDCl_3$)



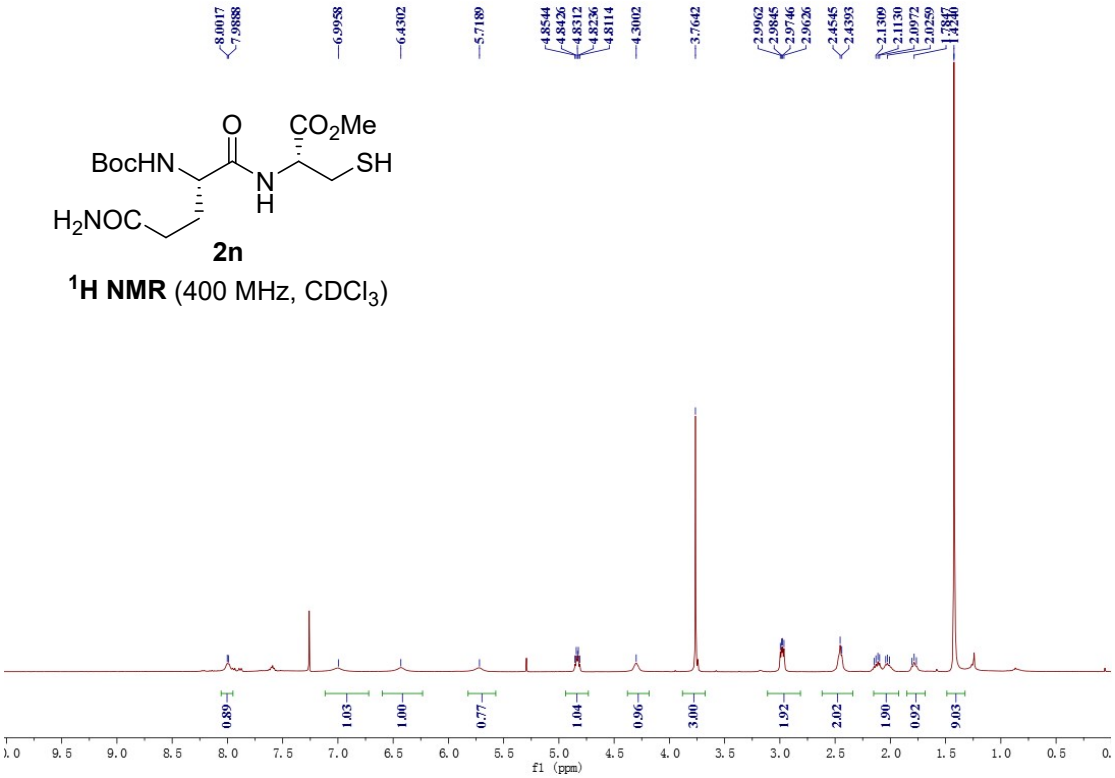
2l

^{1}H NMR (500 MHz, $CDCl_3$)



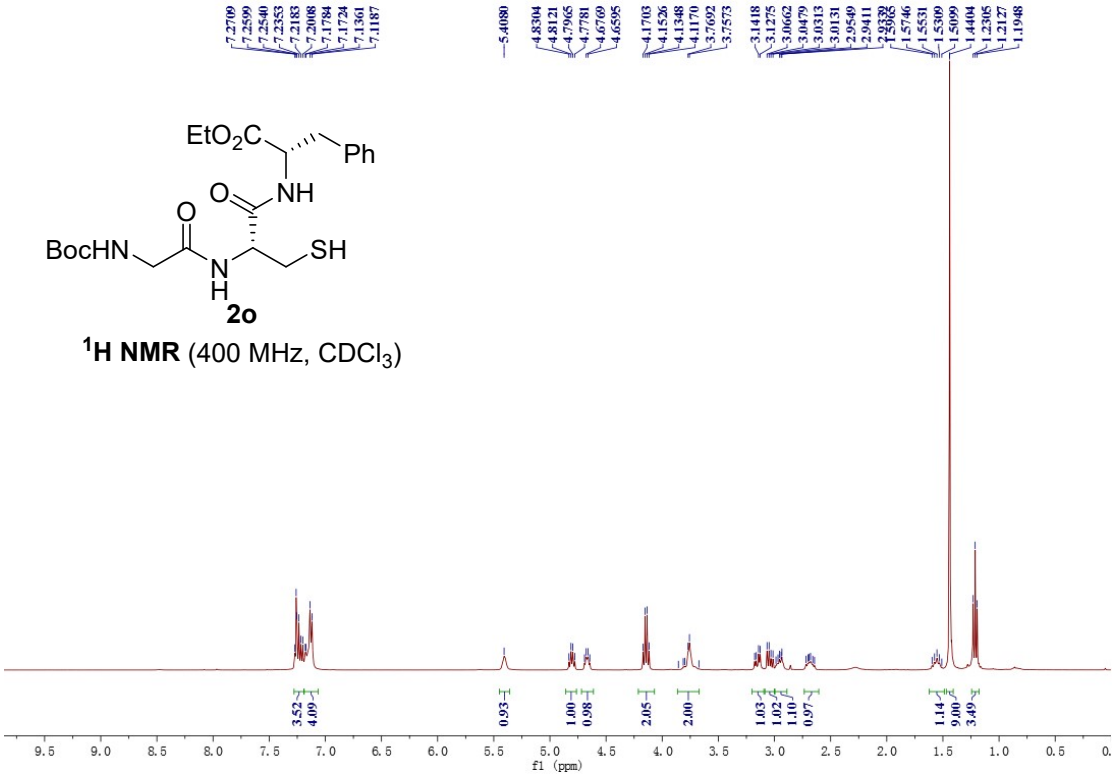
2m

^{1}H NMR (400 MHz, $CDCl_3$)



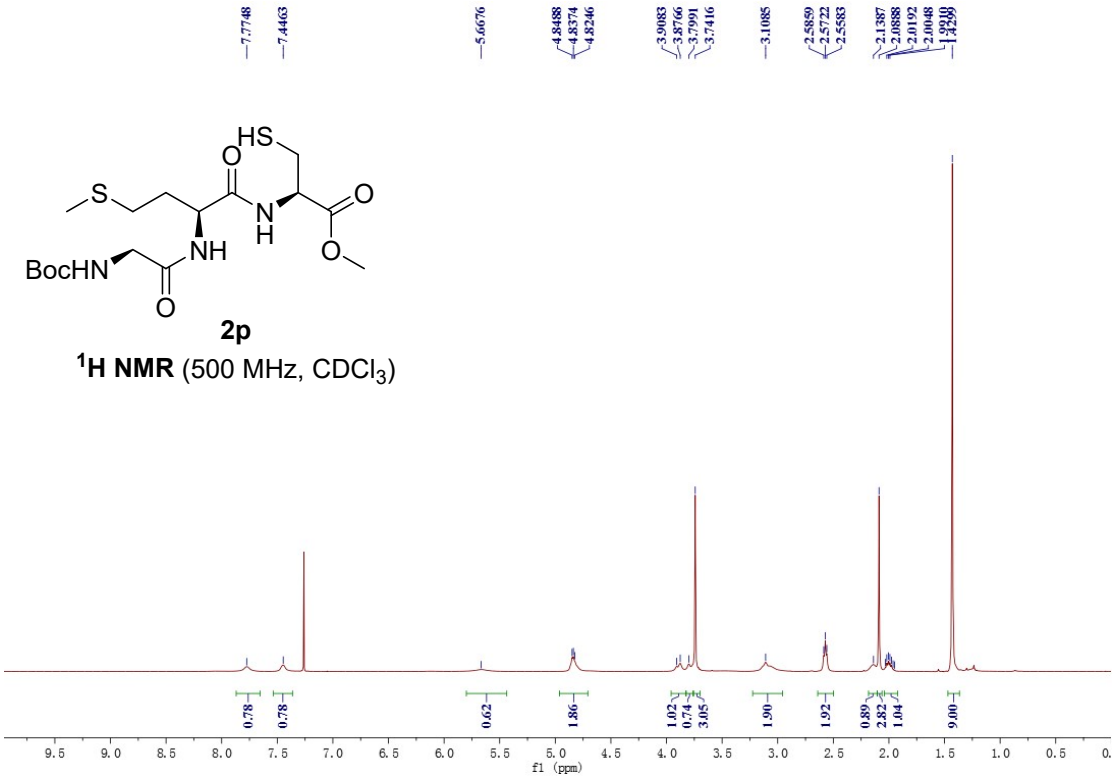
2n

^{1}H NMR (400 MHz, $CDCl_3$)



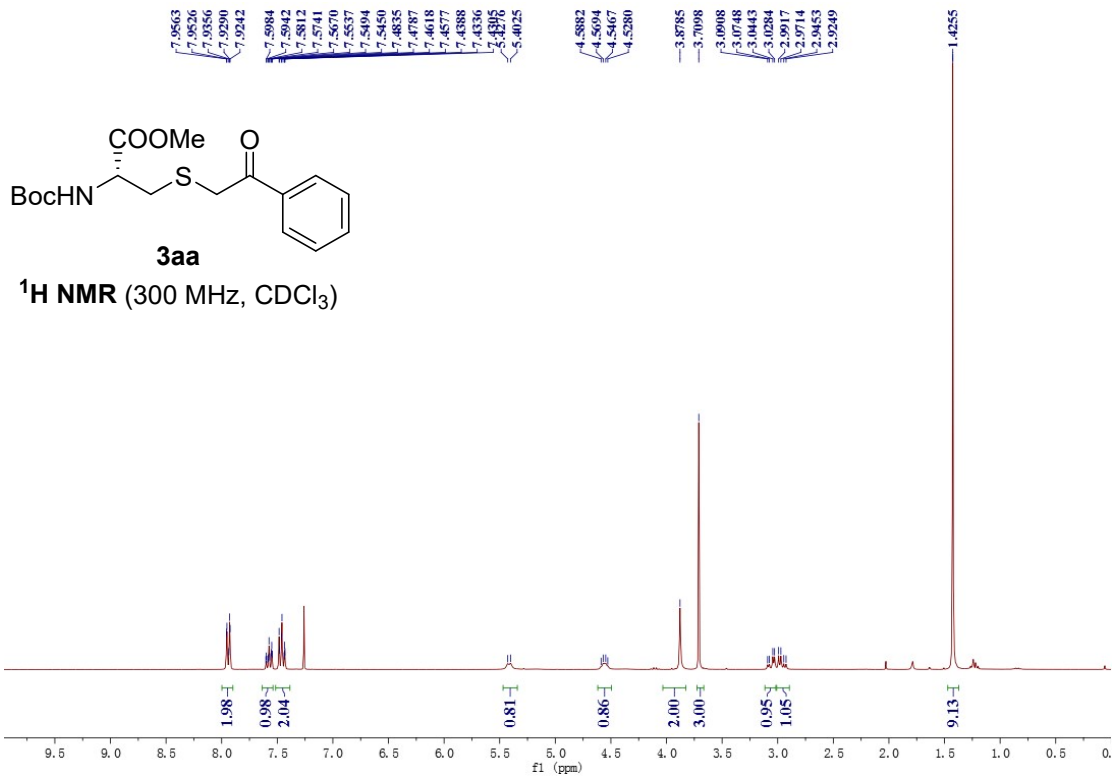
2o

^{1}H NMR (400 MHz, $CDCl_3$)



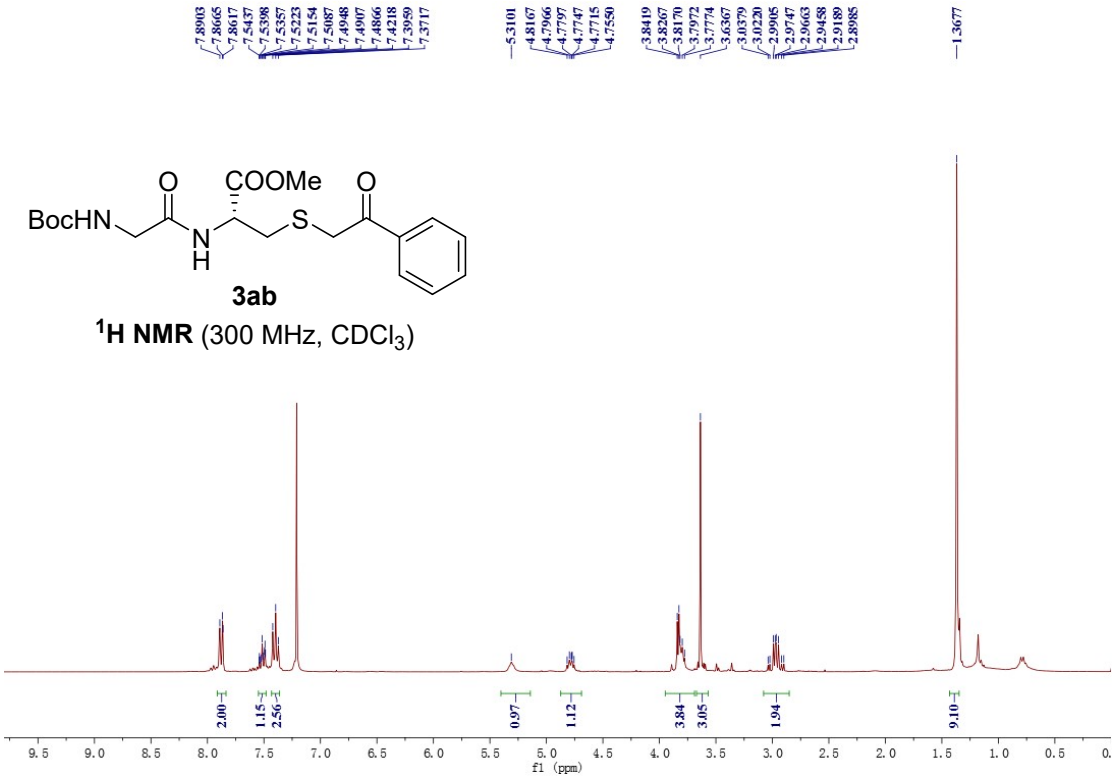
2p

^{1}H NMR (500 MHz, $CDCl_3$)



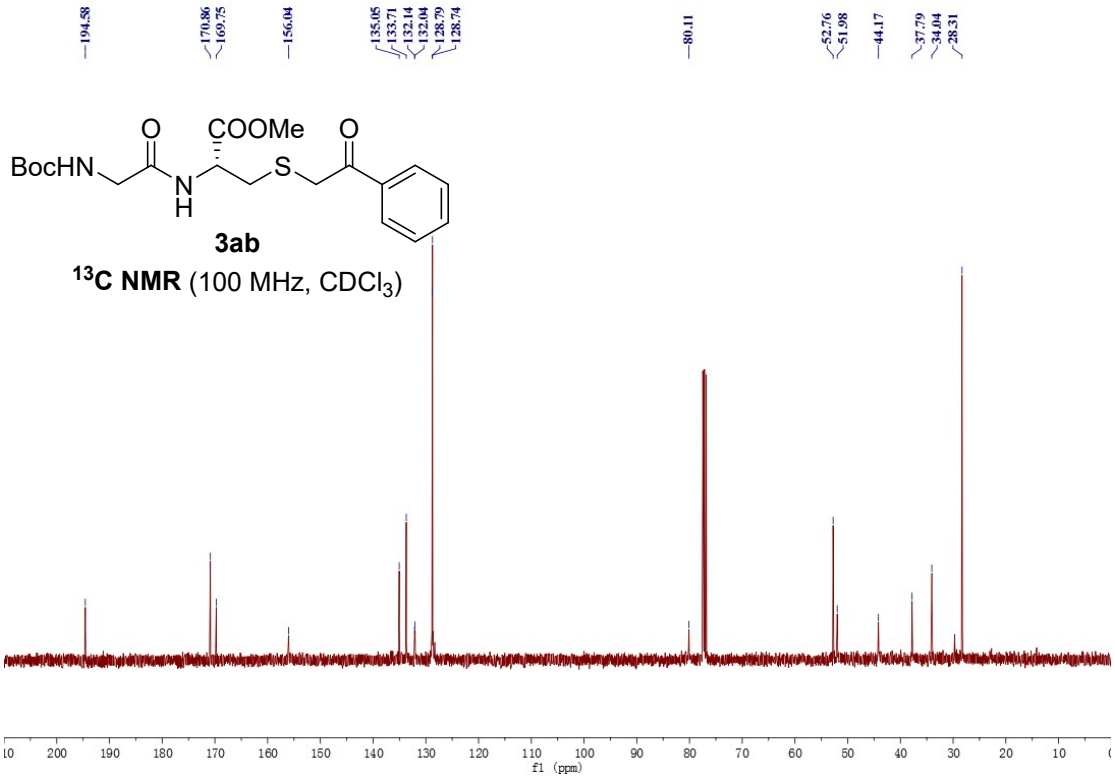
3aa

^{1}H NMR (300 MHz, $CDCl_3$)



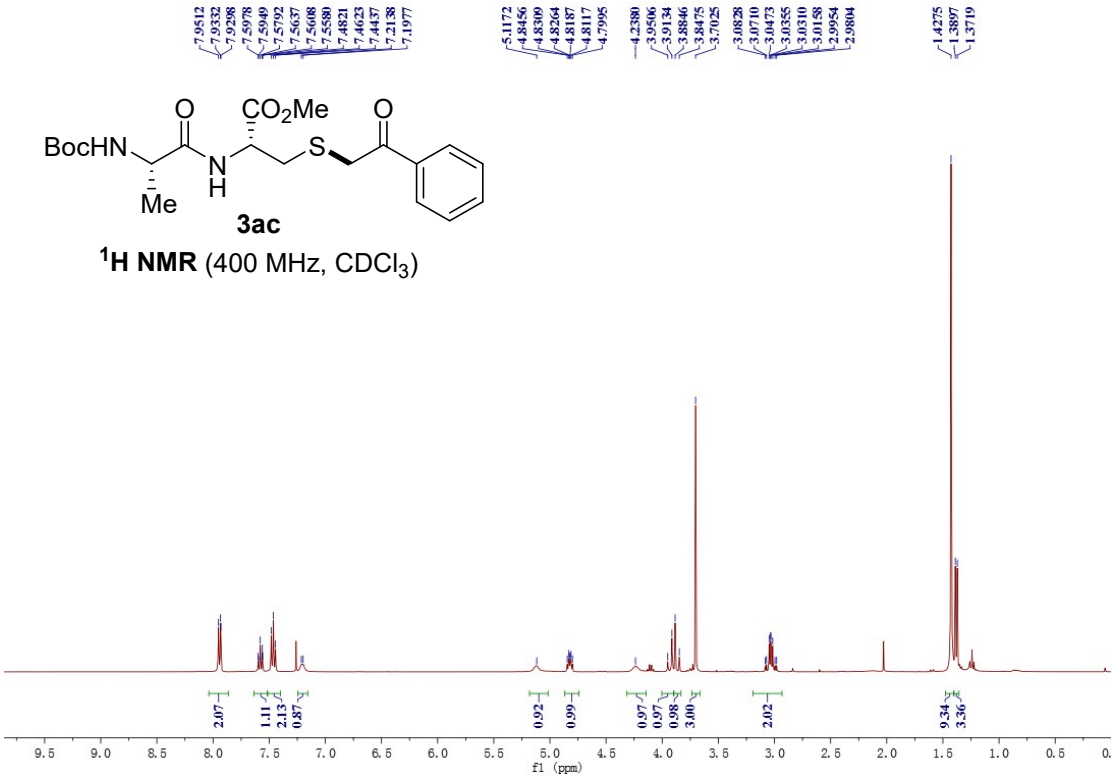
3ab

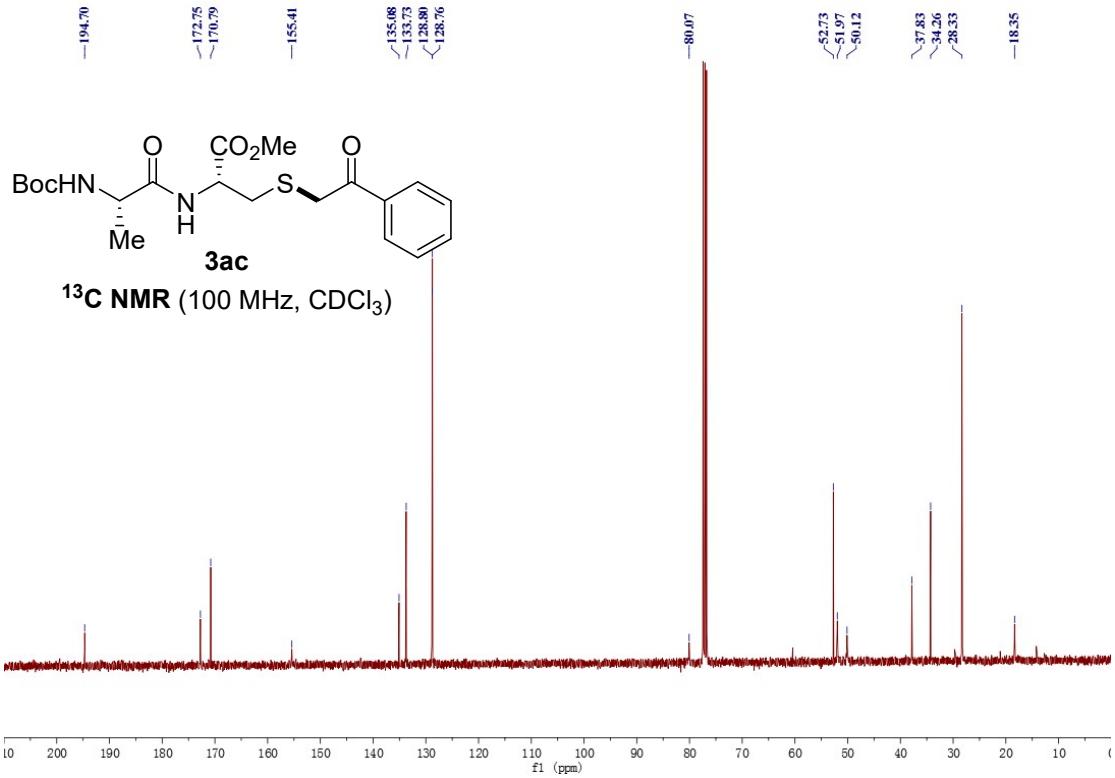
^{1}H NMR (300 MHz, $CDCl_3$)

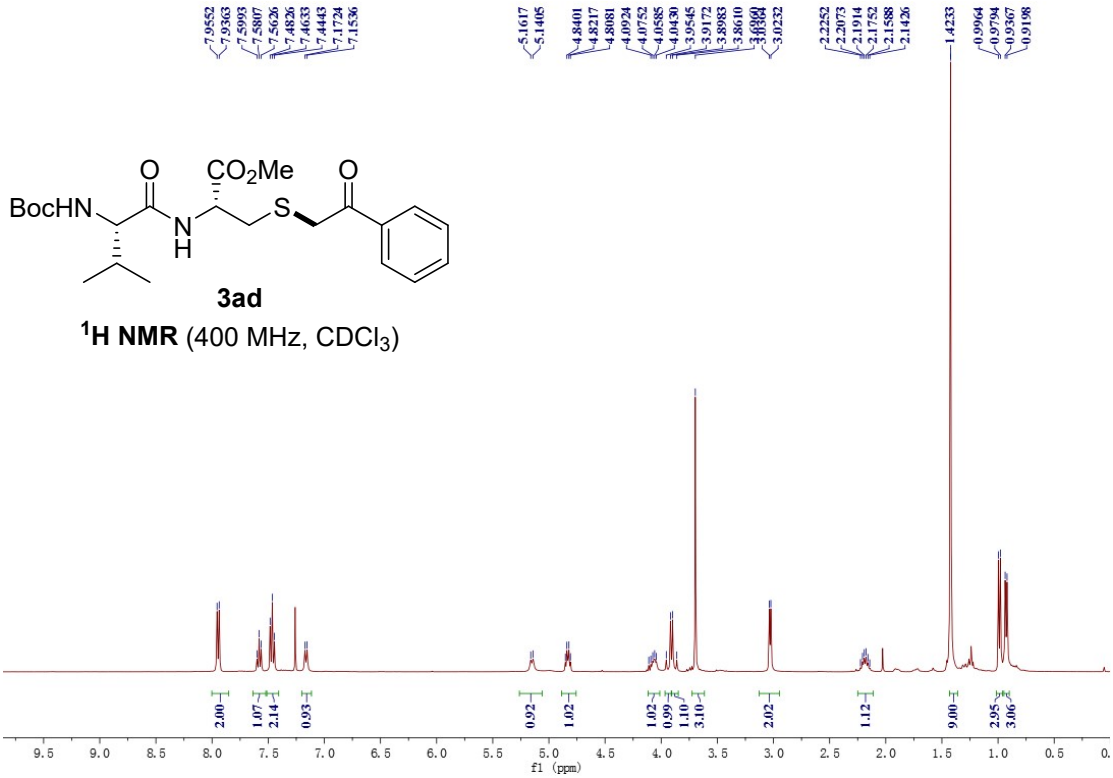


3ab

^{13}C NMR (100 MHz, $CDCl_3$)

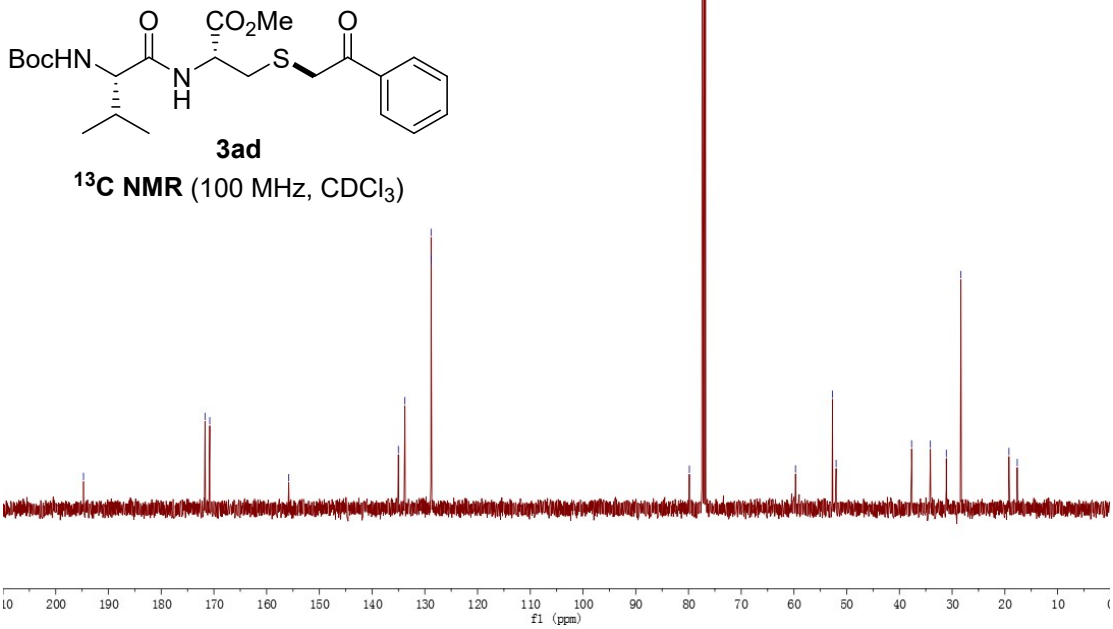

7.9512
7.9332
7.9298
7.5978
7.5949
7.5792
7.5637
7.5608
7.5580
7.4821
7.4623
7.4437
7.2138
7.1977
5.1172
4.8456
4.8309
4.8264
4.8187
4.8117
4.7995
4.2380
3.9506
3.9134
3.8846
3.8475
3.7025
3.0828
3.0710
3.0473
3.0355
3.0310
3.0158
2.9954
2.9804
1.4275
1.3897
1.3719
BocHN
O
CO2Me
O
N
H
S
Me
3ac
1H NMR (400 MHz, CDCl3)
2.07
1.11
2.13
0.87
0.92
0.99
0.97
0.97
0.98
3.00
2.02
9.34
3.36
f1 (ppm)


194.70
172.75
170.79
155.41
135.08
133.73
128.80
128.76
80.07
52.73
51.97
50.12
37.83
34.26
28.33
18.35
BocHN
O
CO2Me
O
N
H
S
Me
3ac
13C NMR (100 MHz, CDCl3)
f1 (ppm)



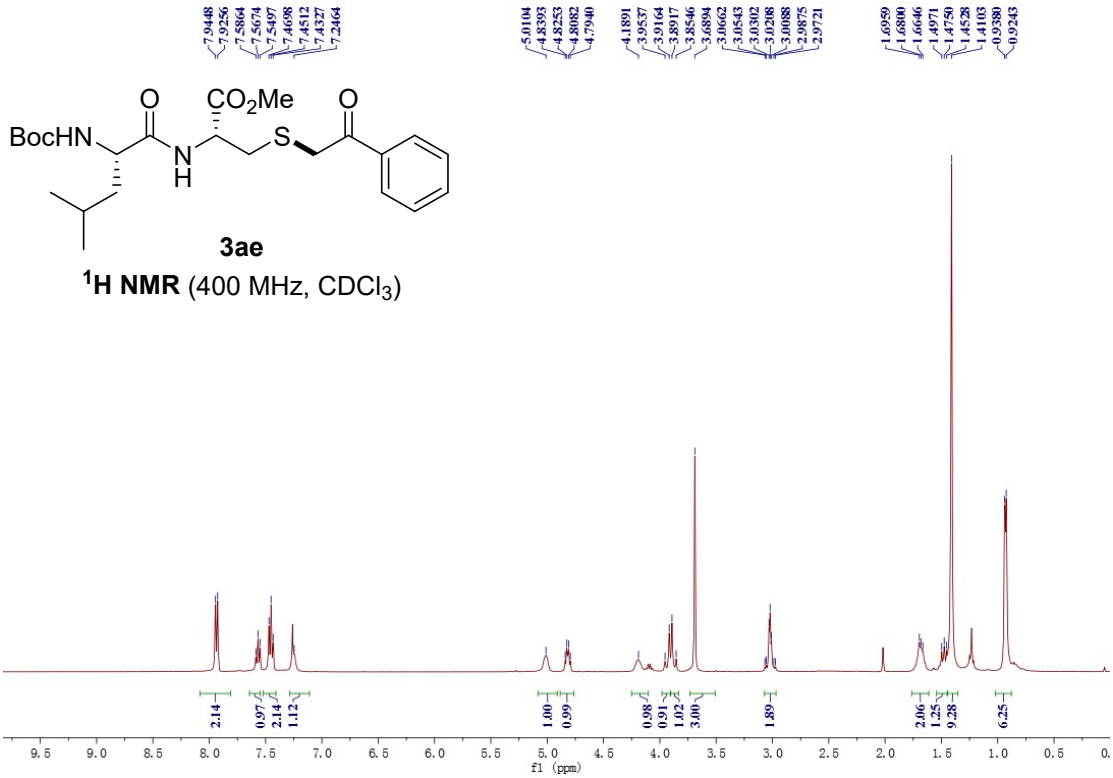
3ad

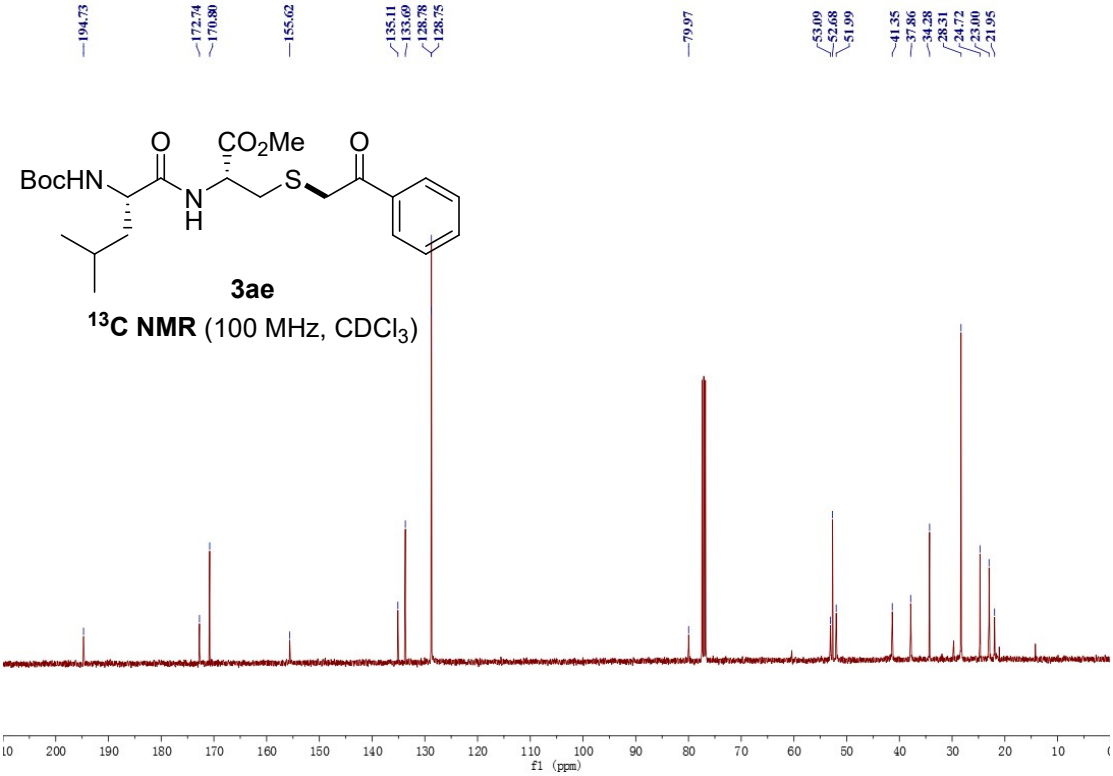
^{1}H NMR (400 MHz, $CDCl_3$)

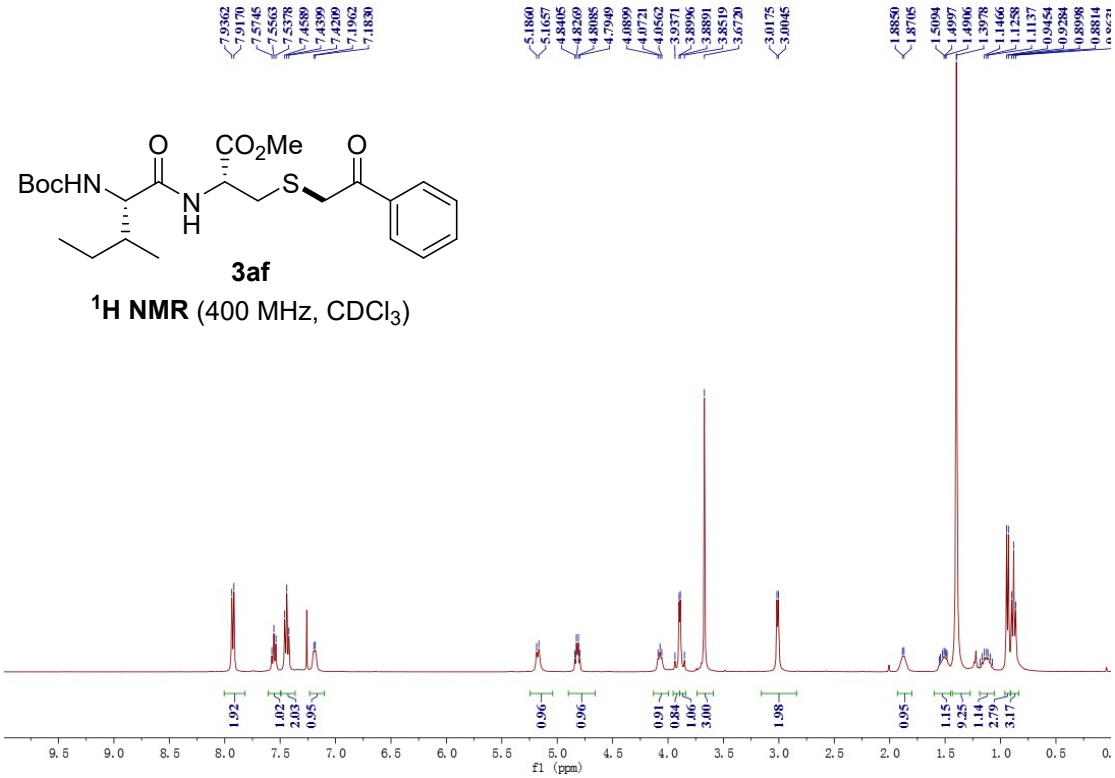


3ad

^{13}C NMR (100 MHz, $CDCl_3$)

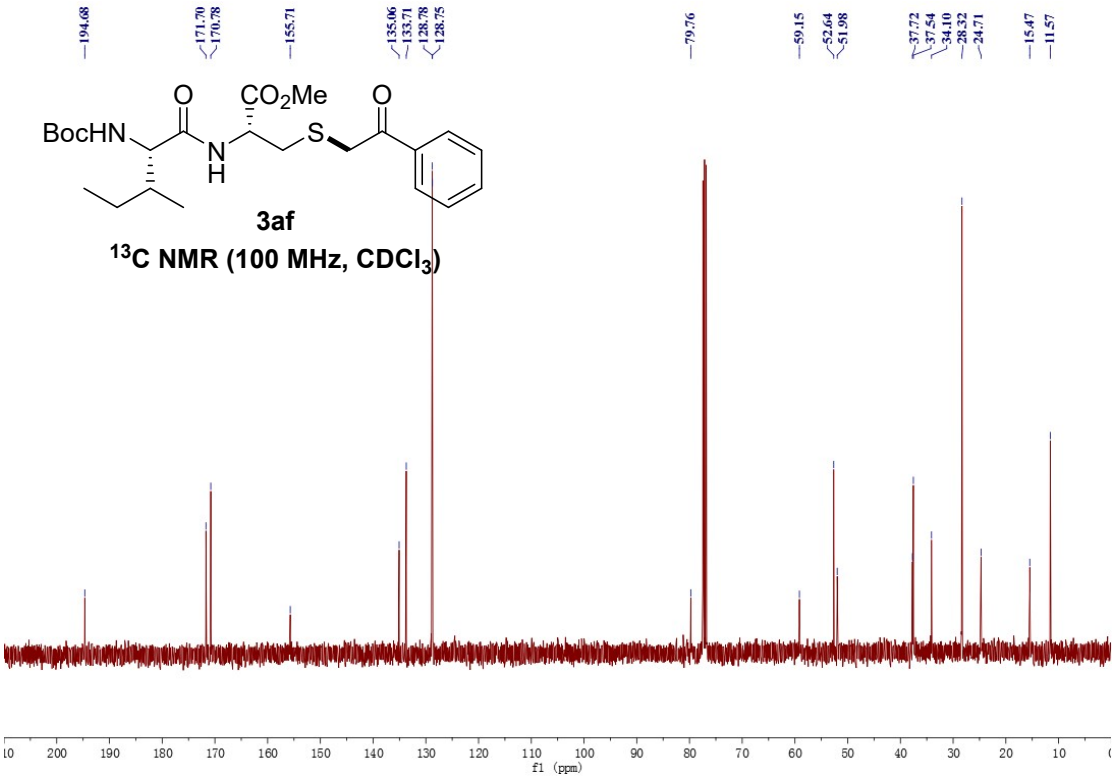

7.9448
7.9256
7.5864
7.5674
7.5497
7.4698
7.4512
7.4327
7.2464
5.0104
4.8393
4.8253
4.8082
4.7940
4.1891
3.9537
3.9164
3.8917
3.8546
3.6894
3.0662
3.0543
3.0302
3.0208
3.0088
2.9875
2.9721
1.6959
1.6800
1.6646
1.4971
1.4750
1.4528
1.4103
0.9380
0.9243
BocHN
O
CO2Me
O
N
H
S
3ae
1H NMR (400 MHz, CDCl3)
2.14
0.97
2.14
1.12
1.00
0.99
0.98
0.91
1.02
3.00
1.89
2.06
1.25
9.28
6.25
f1 (ppm)


194.73
172.74
170.80
155.62
135.11
133.69
128.78
128.75
79.97
53.09
52.68
51.99
41.35
37.86
34.28
28.31
24.72
23.00
21.95
BocHN
O
CO2Me
O
N
H
S
3ae
13C NMR (100 MHz, CDCl3)
f1 (ppm)



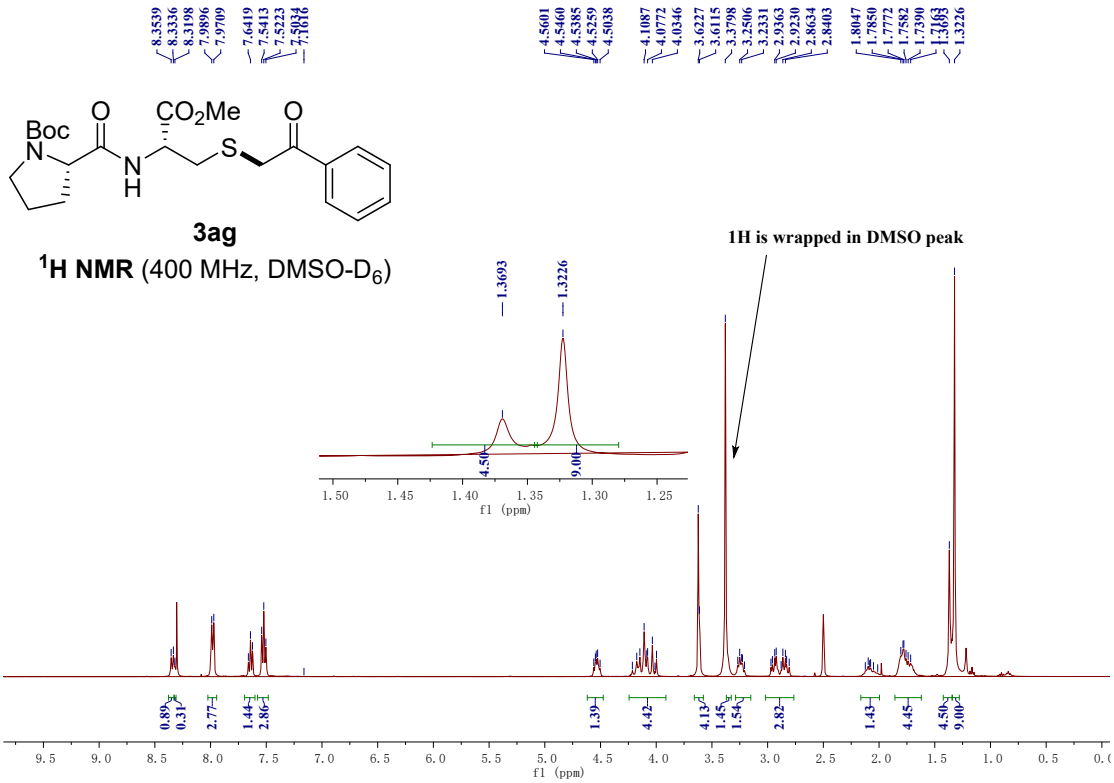
3af

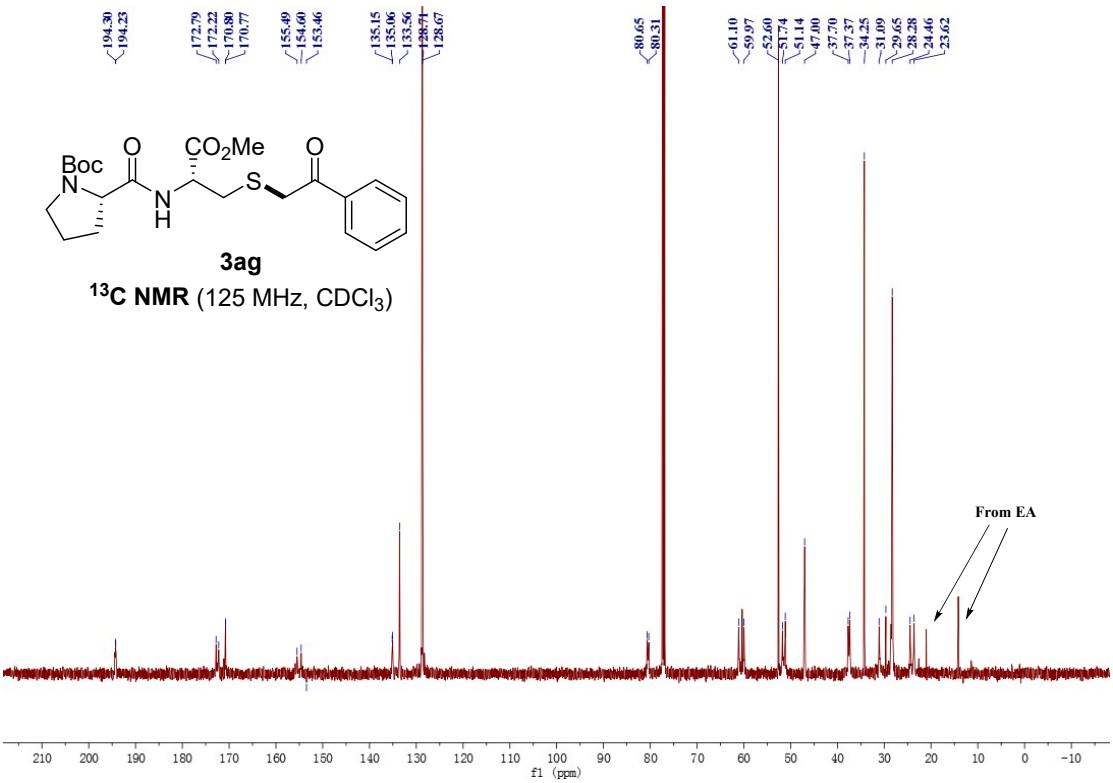
^{1}H NMR (400 MHz, $CDCl_3$)

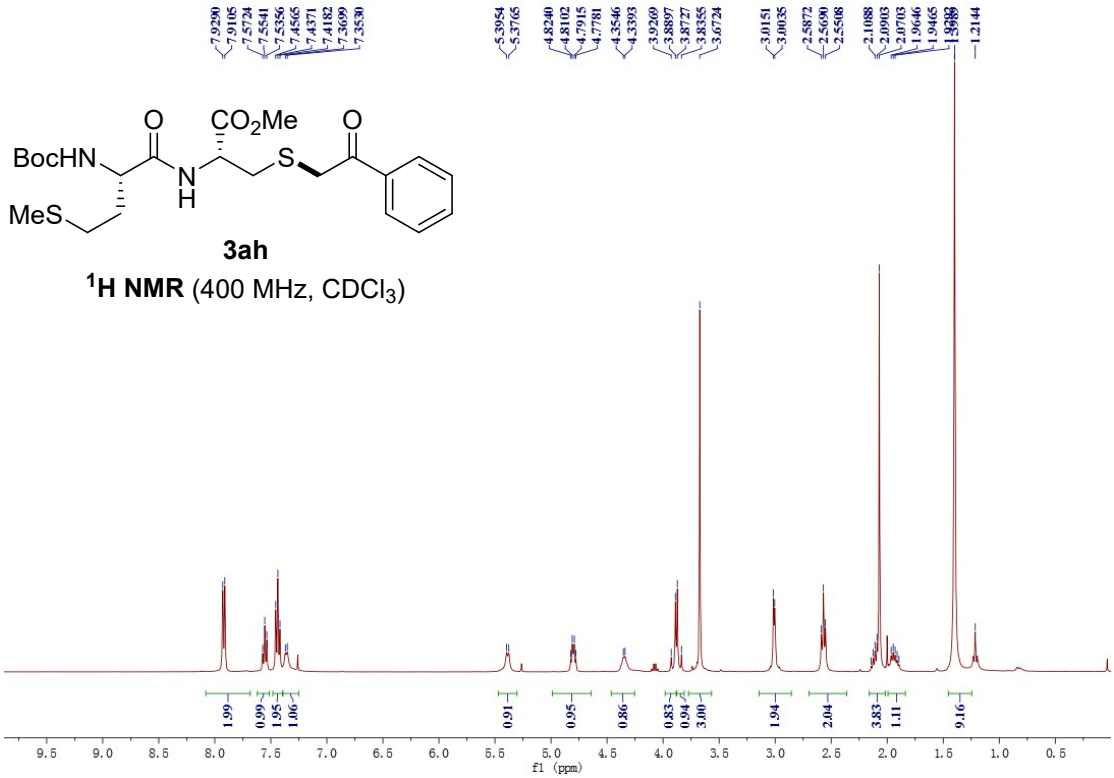


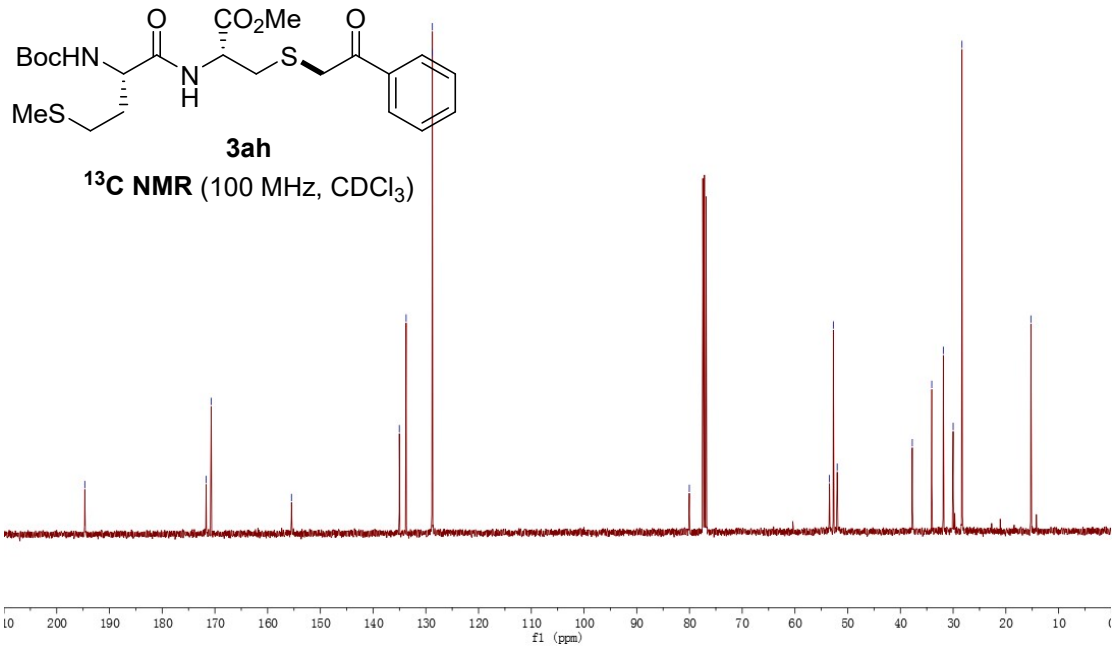
3af

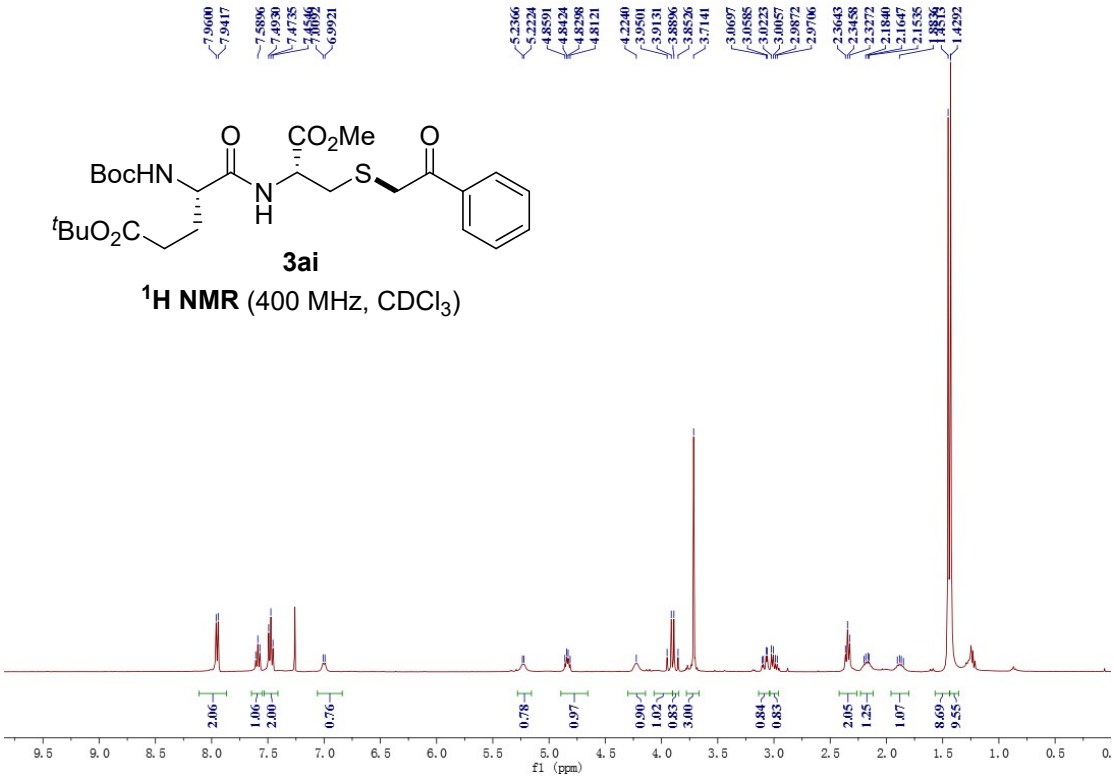
^{13}C NMR (100 MHz, $CDCl_3$)


Boc
CO2Me
3ag
1H NMR (400 MHz, DMSO-D6)
1H is wrapped in DMSO peak


Boc
CO2Me
3ag
13C NMR (125 MHz, CDCl3)
From EA

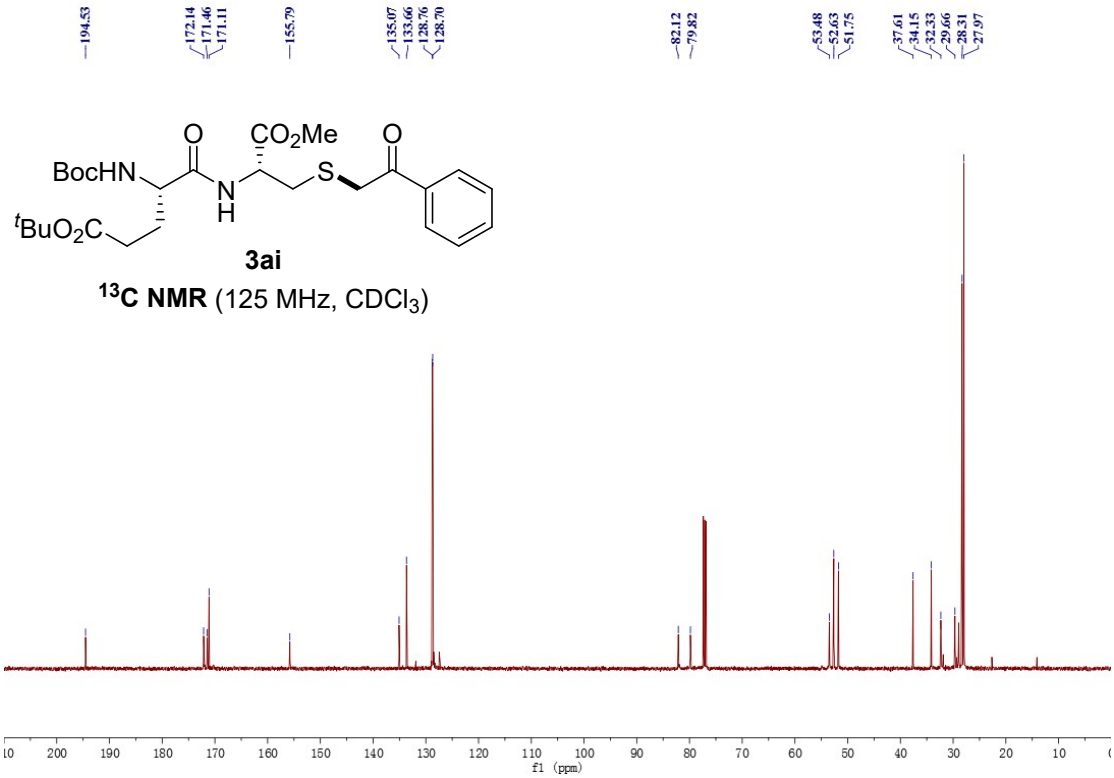

BocHN
O
CO2Me
O
N
H
S
MeS
3ah
1H NMR (400 MHz, CDCl3)
f1 (ppm)


BocHN
O
CO2Me
O
N
H
S
MeS
3ah
13C NMR (100 MHz, CDCl3)
f1 (ppm)



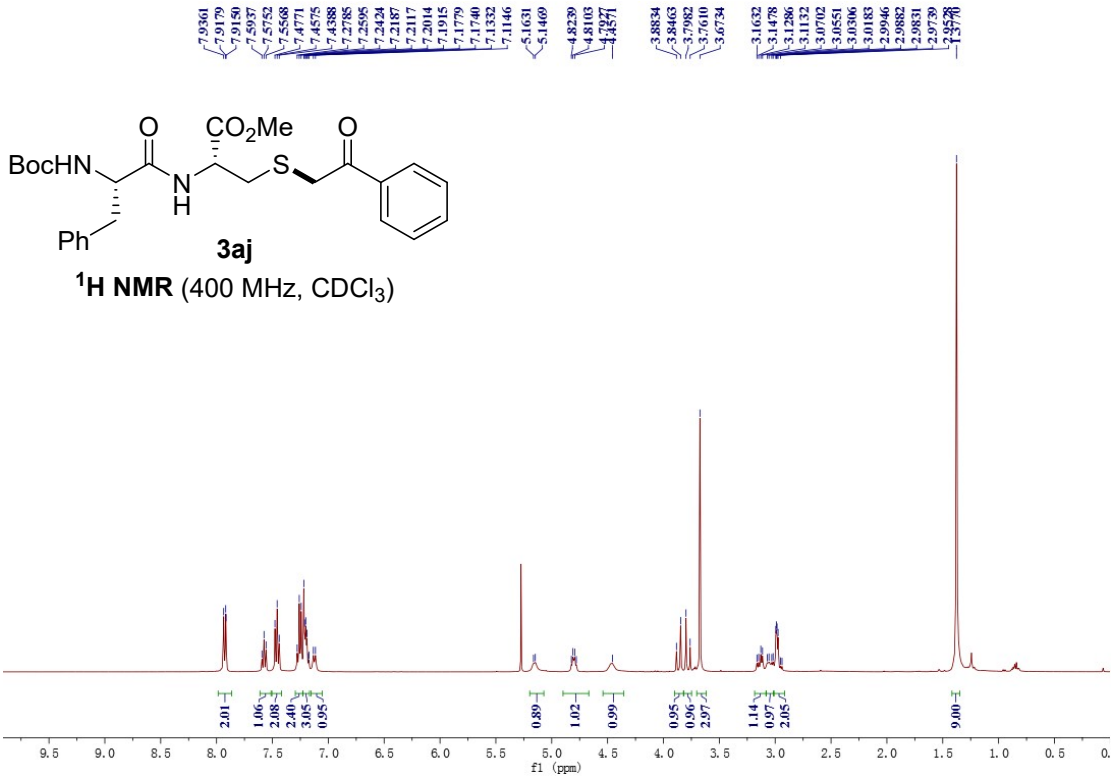
3ai

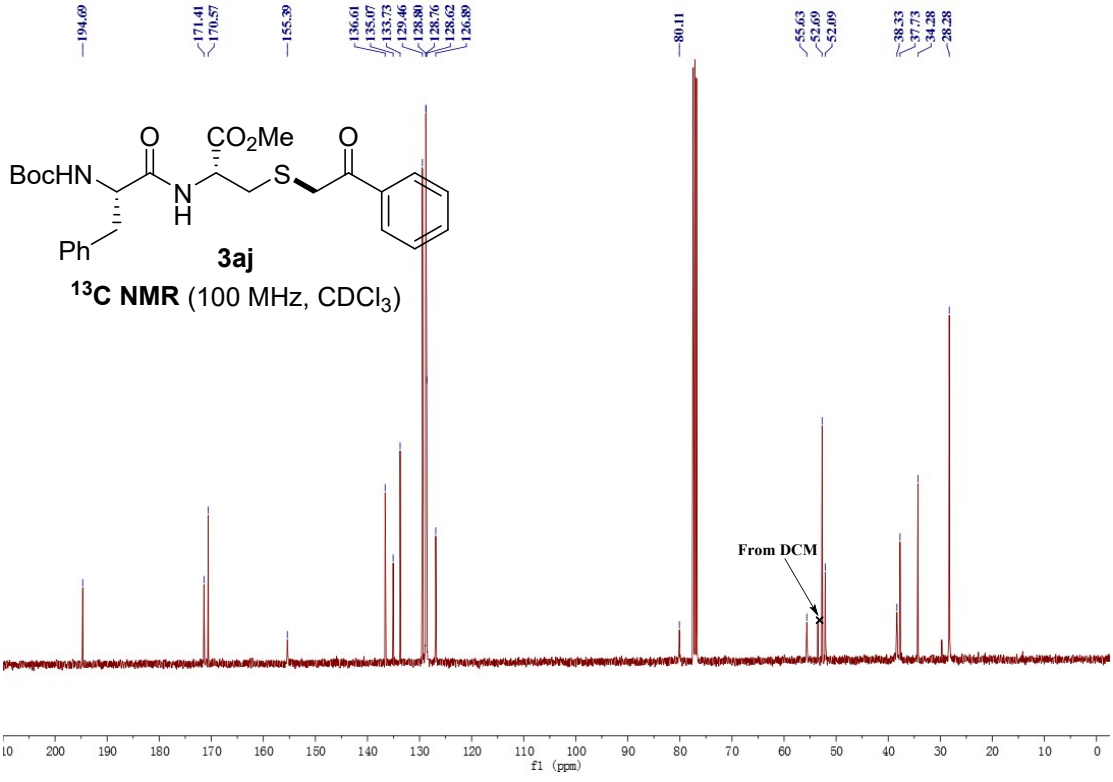
^{1}H NMR (400 MHz, $CDCl_3$)

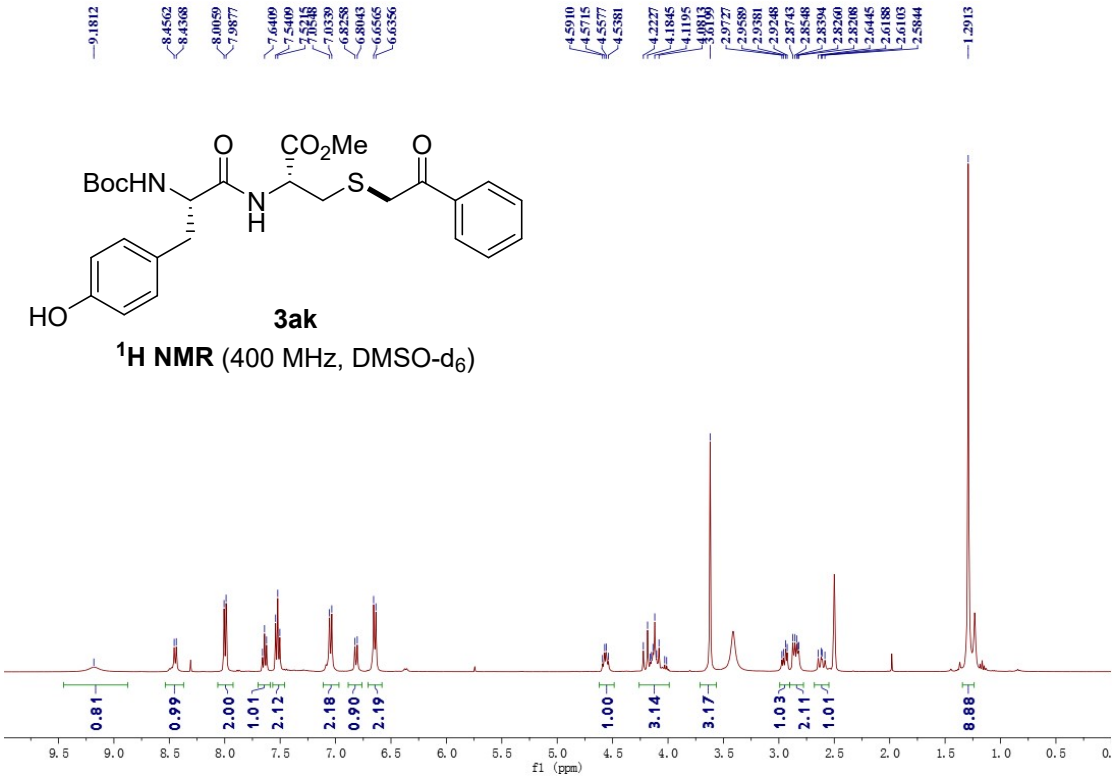


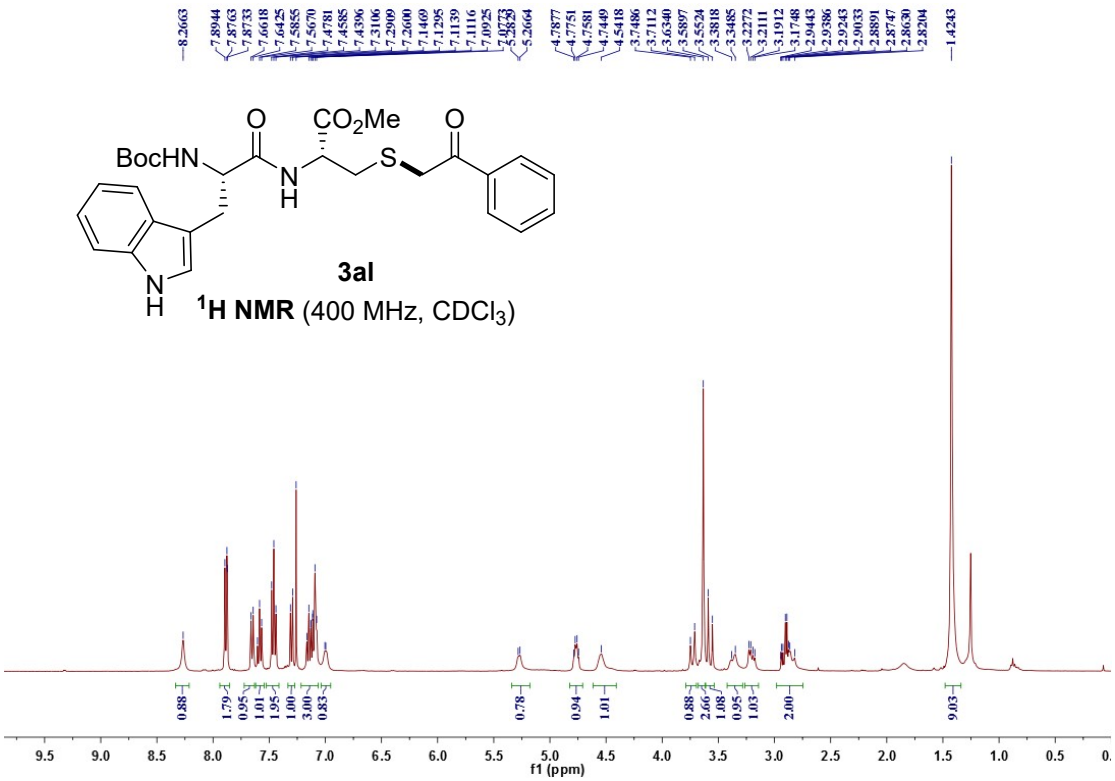
3ai

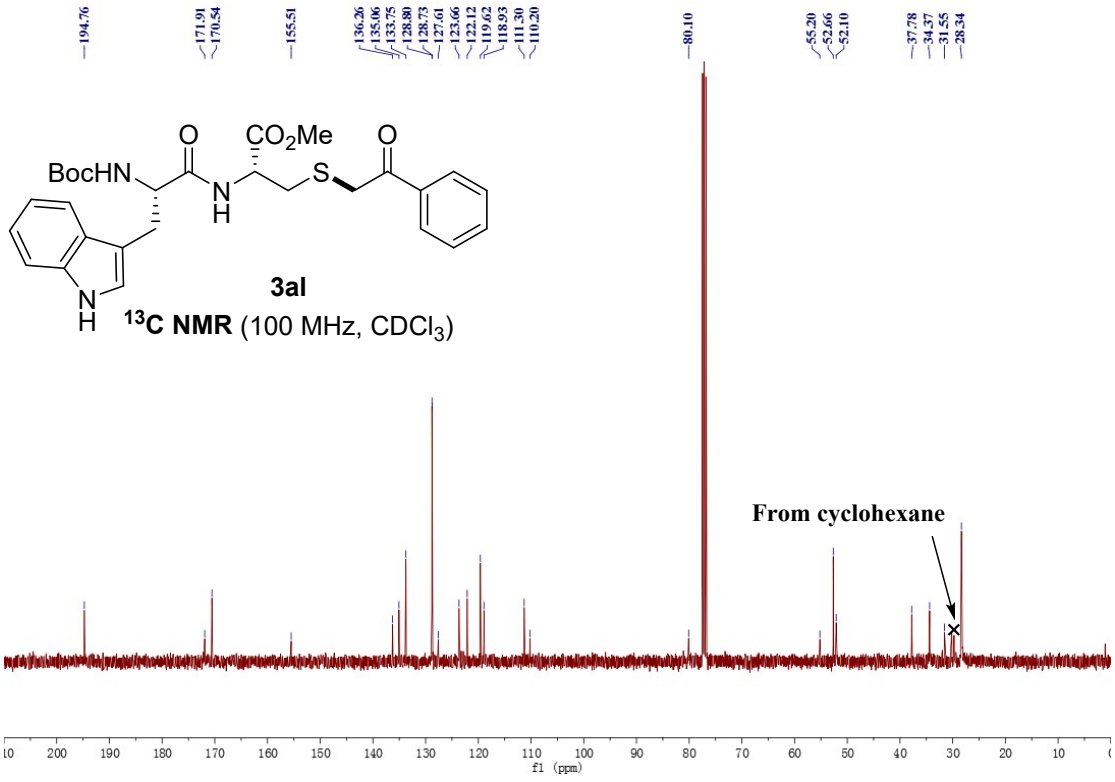
^{13}C NMR (125 MHz, $CDCl_3$)

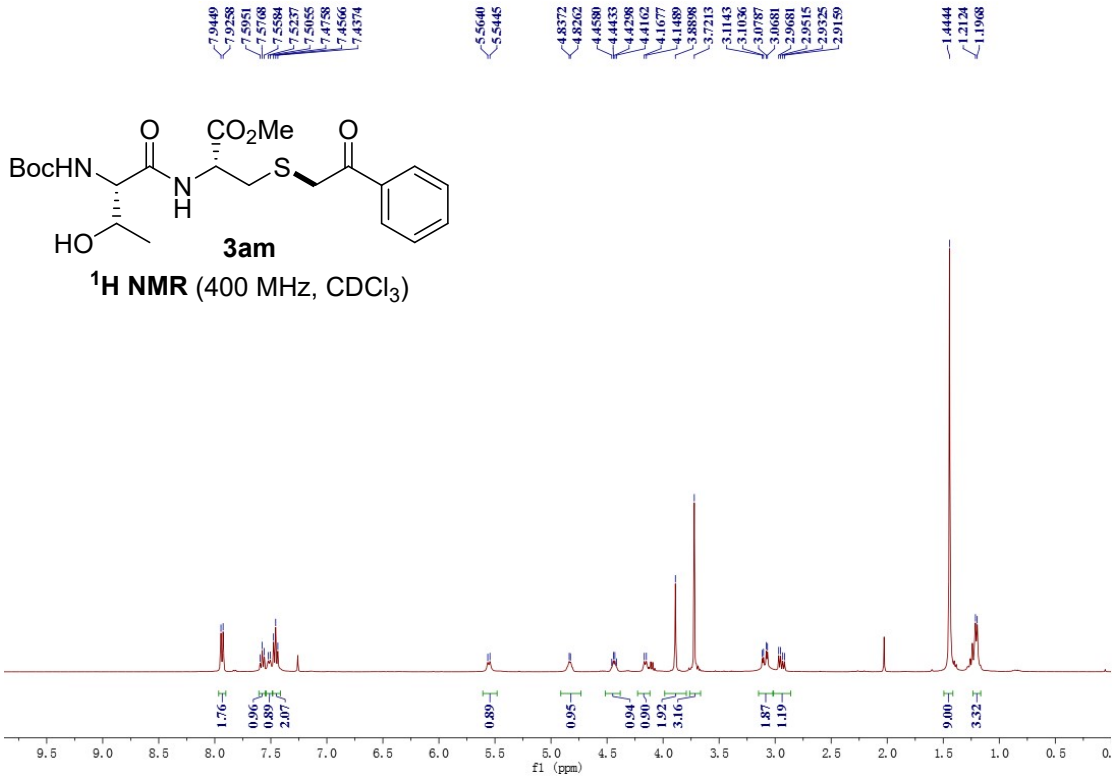

BocHN
O
CO2Me
O
N
H
S
Ph
3aj
1H NMR (400 MHz, CDCl3)
f1 (ppm)

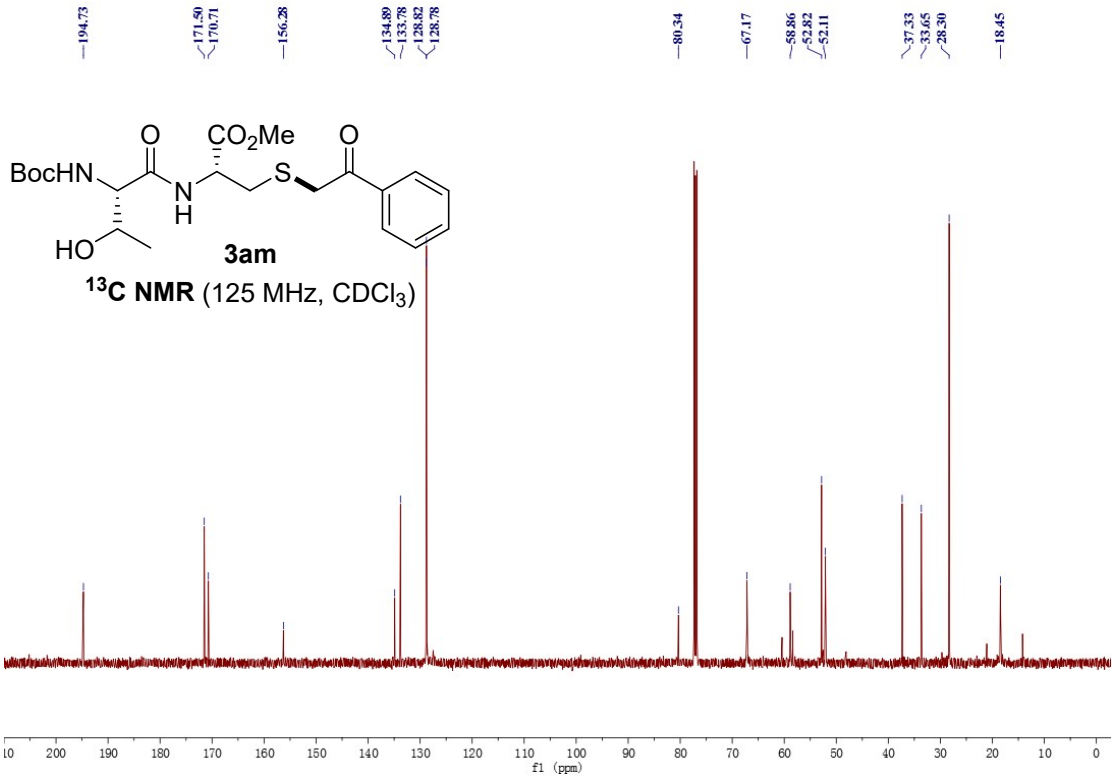

BocHN
O
CO2Me
O
N
H
S
Ph
3aj
13C NMR (100 MHz, CDCl3)
From DCM
f1 (ppm)

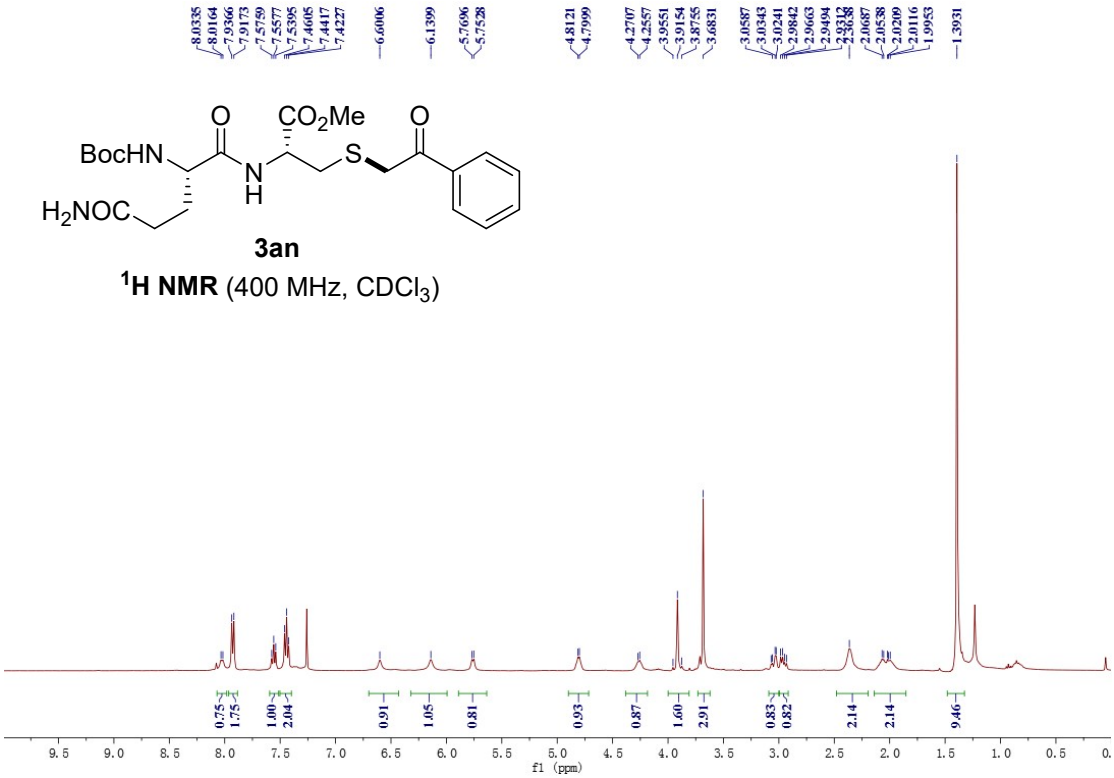

BocHN
O
CO2Me
O
N
H
S
O
HO
3ak
1H NMR (400 MHz, DMSO-d6)
f1 (ppm)
13C NMR (100 MHz, DMSO-d6)
f1 (ppm)


BocHN
O
CO2Me
O
N
H
S
O
N
H
3al
1H NMR (400 MHz, CDCl3)
f1 (ppm)


BocHN
O
CO2Me
O
N
H
S
N
H
3al
13C NMR (100 MHz, CDCl3)
From cyclohexane
f1 (ppm)

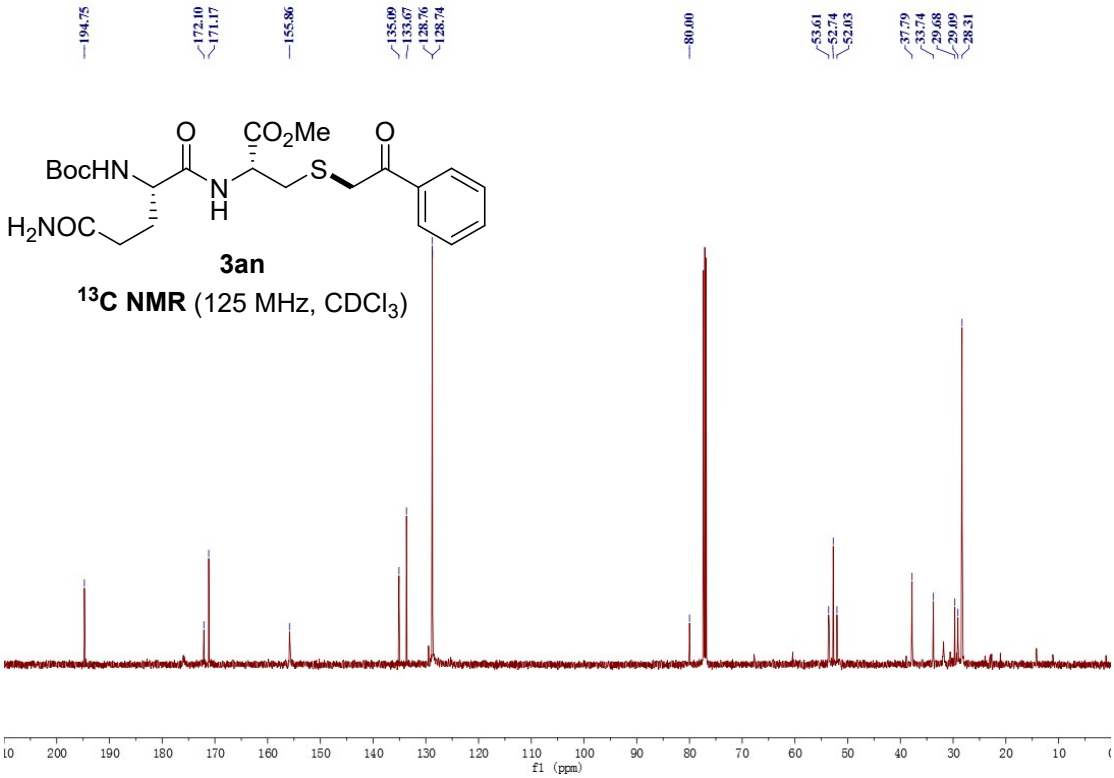

7.9449
7.9258
7.5951
7.5768
7.5584
7.5237
7.5055
7.4758
7.4566
7.4374
5.5640
5.5445
4.8372
4.8262
4.4580
4.4433
4.4298
4.4162
4.1677
4.1489
3.8898
3.7213
3.1143
3.1036
3.0787
3.0681
2.9681
2.9515
2.9325
2.9159
1.4444
1.2124
1.1968
BocHN
O
CO2Me
O
N
H
S
HO
3am
1H NMR (400 MHz, CDCl3)
1.76
0.96
0.89
2.07
0.89
0.95
0.94
0.90
1.92
3.16
1.87
1.19
9.00
3.32
f1 (ppm)


194.73
171.50
170.71
156.28
134.89
133.78
128.82
128.78
80.34
67.17
58.86
52.82
52.11
37.33
33.65
28.30
18.45
BocHN
O
CO2Me
O
N
H
S
HO
3am
13C NMR (125 MHz, CDCl3)
f1 (ppm)



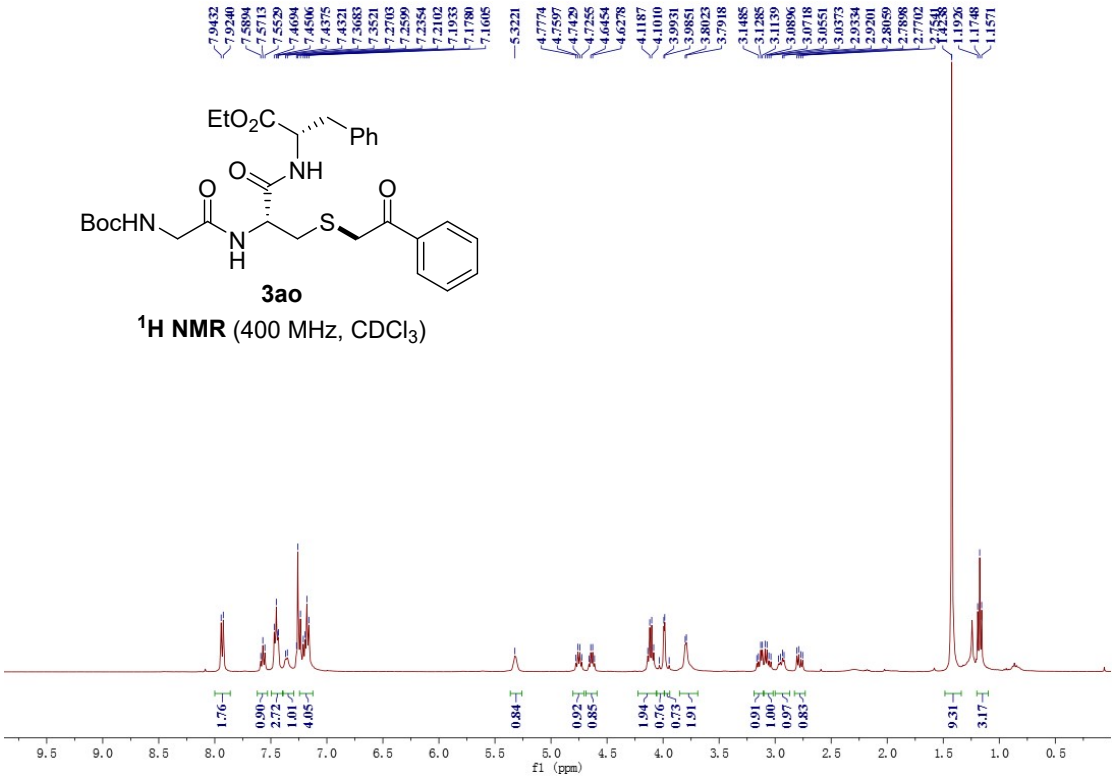
3an

^{1}H NMR (400 MHz, $CDCl_3$)

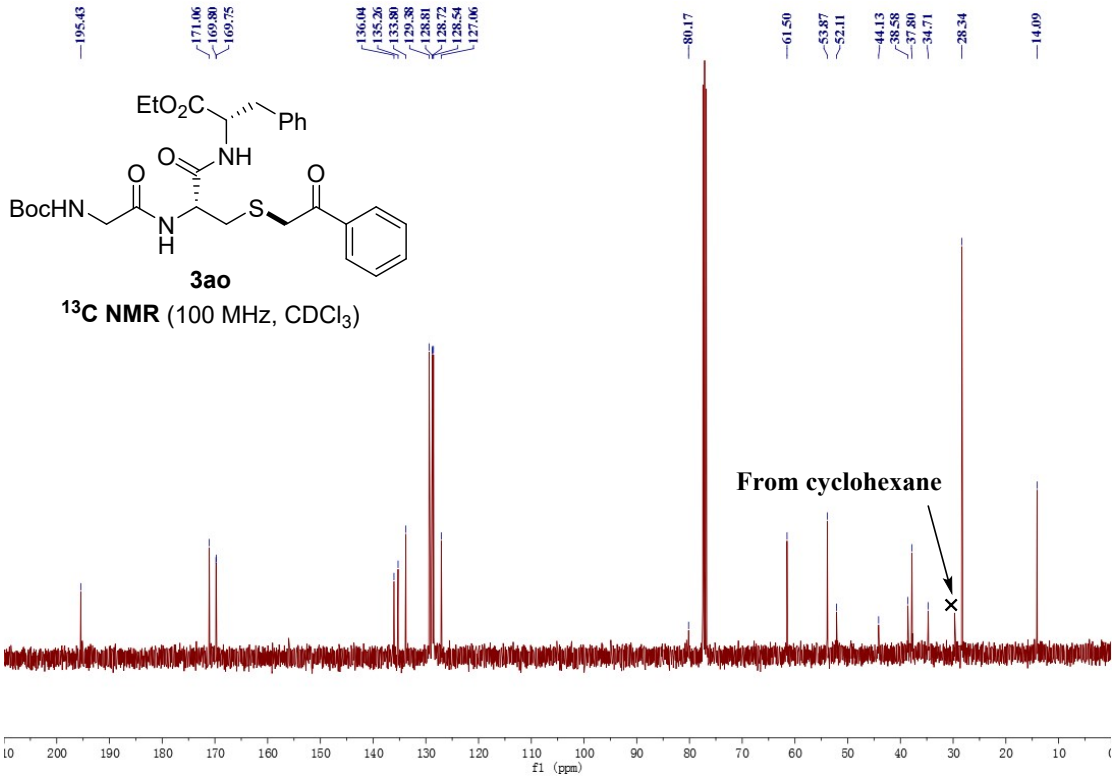


3an

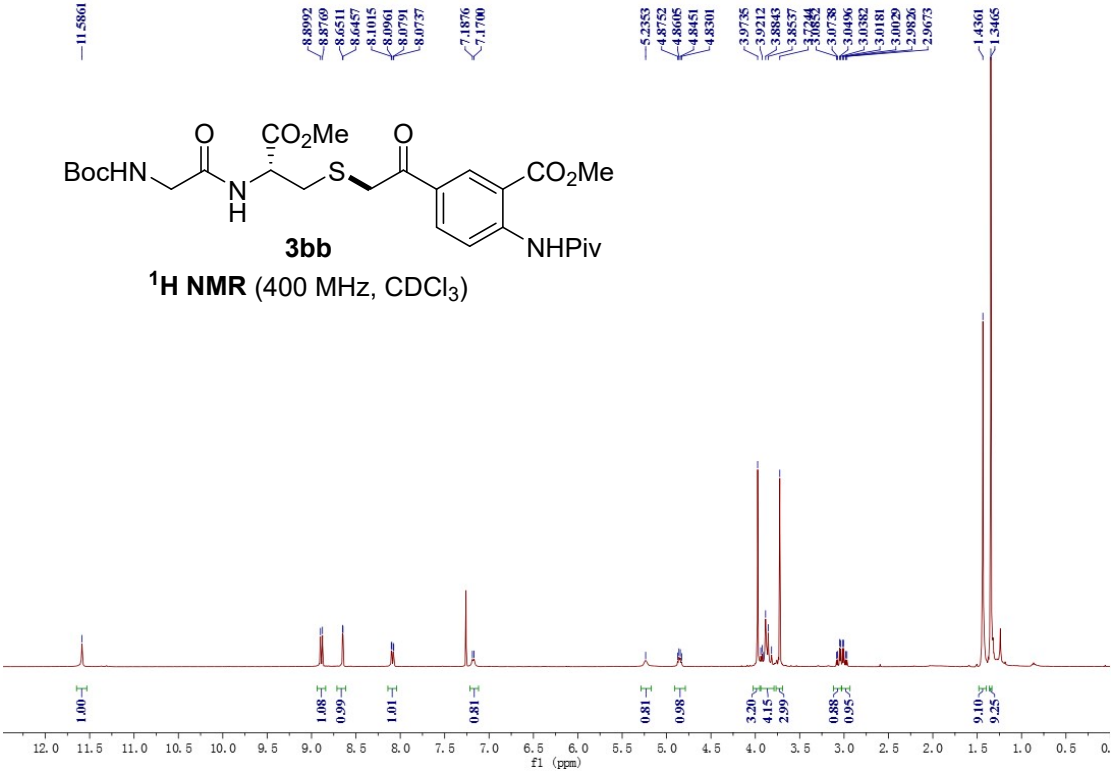
^{13}C NMR (125 MHz, $CDCl_3$)



^{1}H NMR (400 MHz, $CDCl_3$)

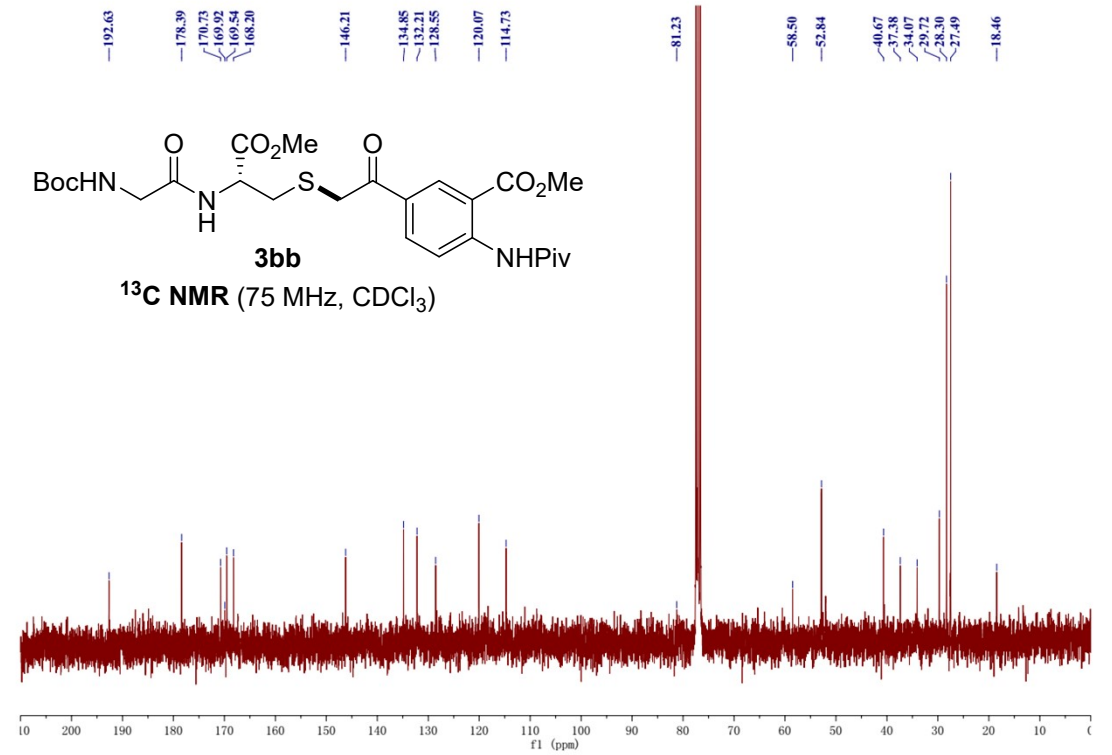


^{13}C NMR (100 MHz, $CDCl_3$)



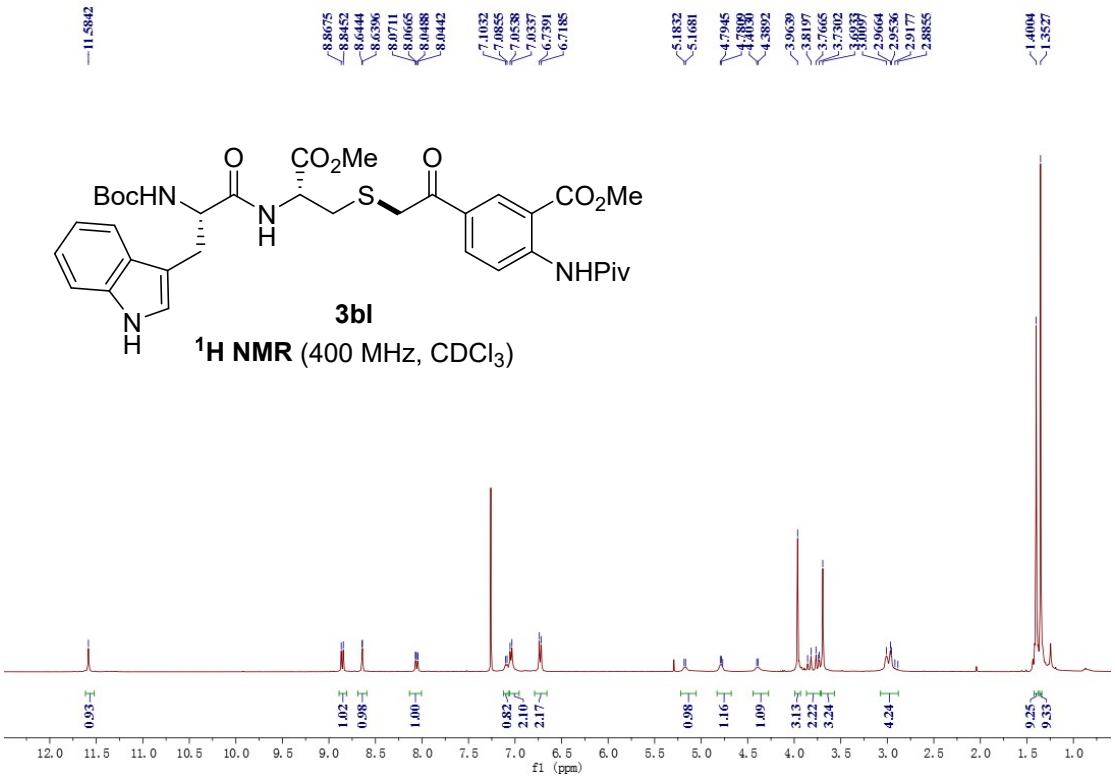
3bb

^{1}H NMR (400 MHz, $CDCl_3$)



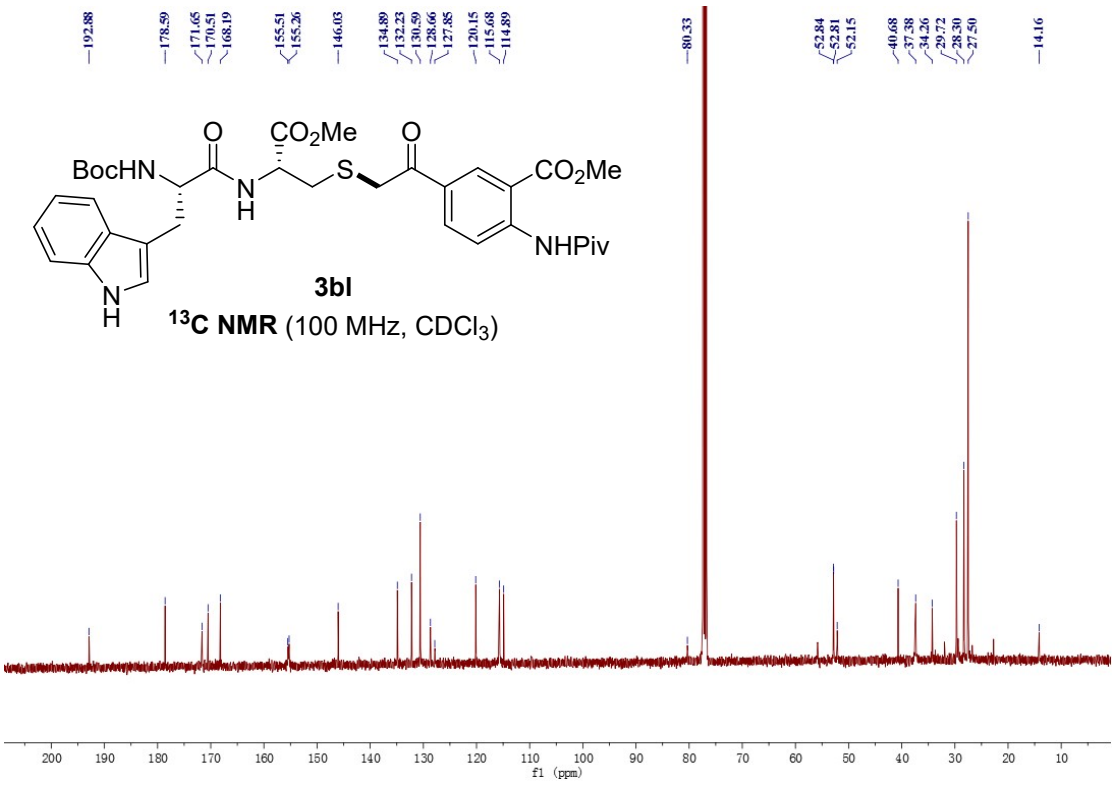
3bb

^{13}C NMR (75 MHz, $CDCl_3$)



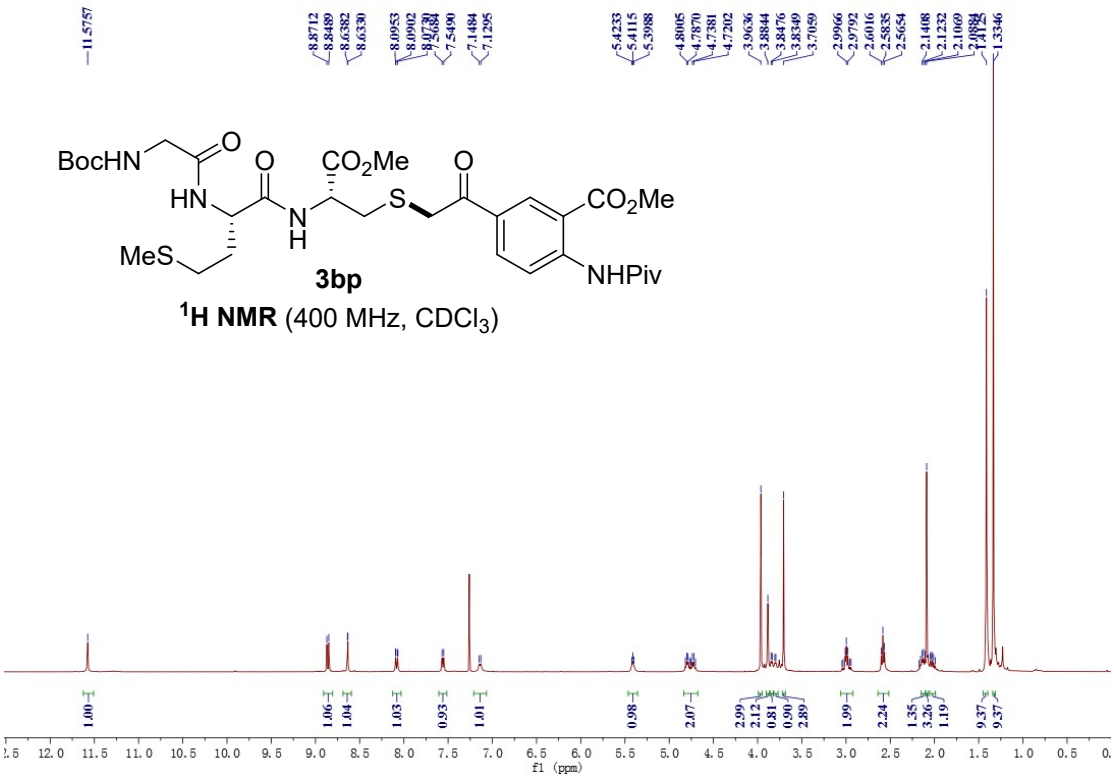
3bl

^{1}H NMR (400 MHz, $CDCl_3$)

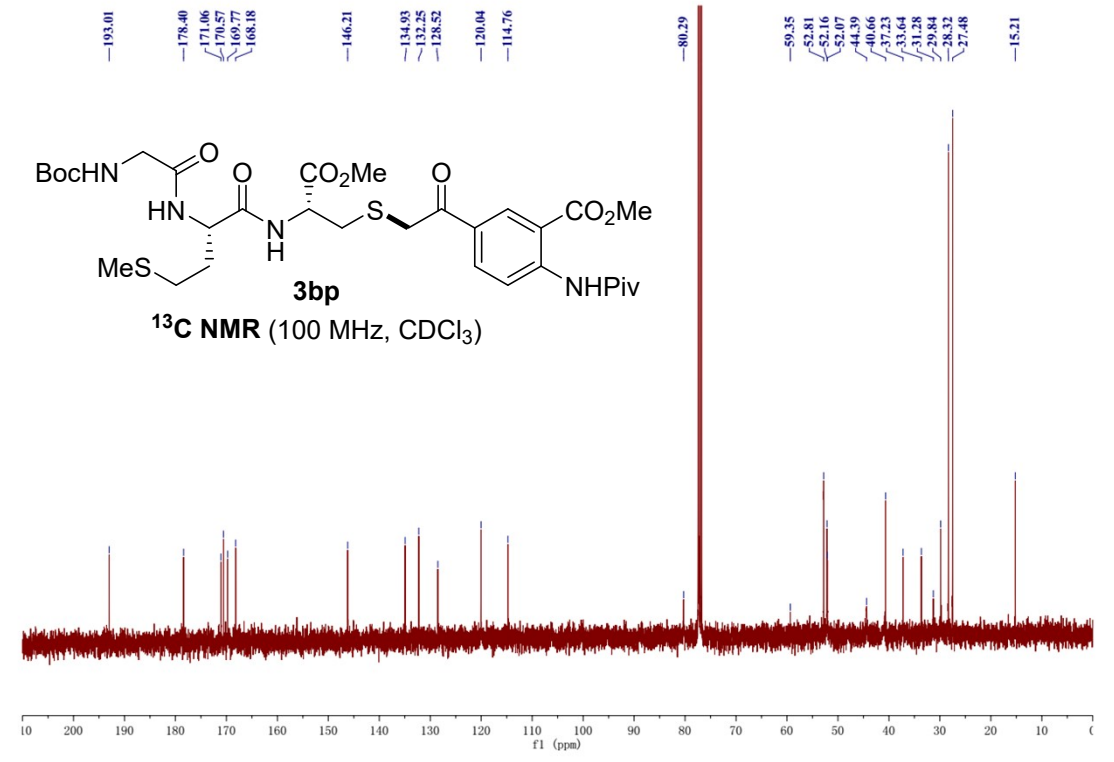


3bl

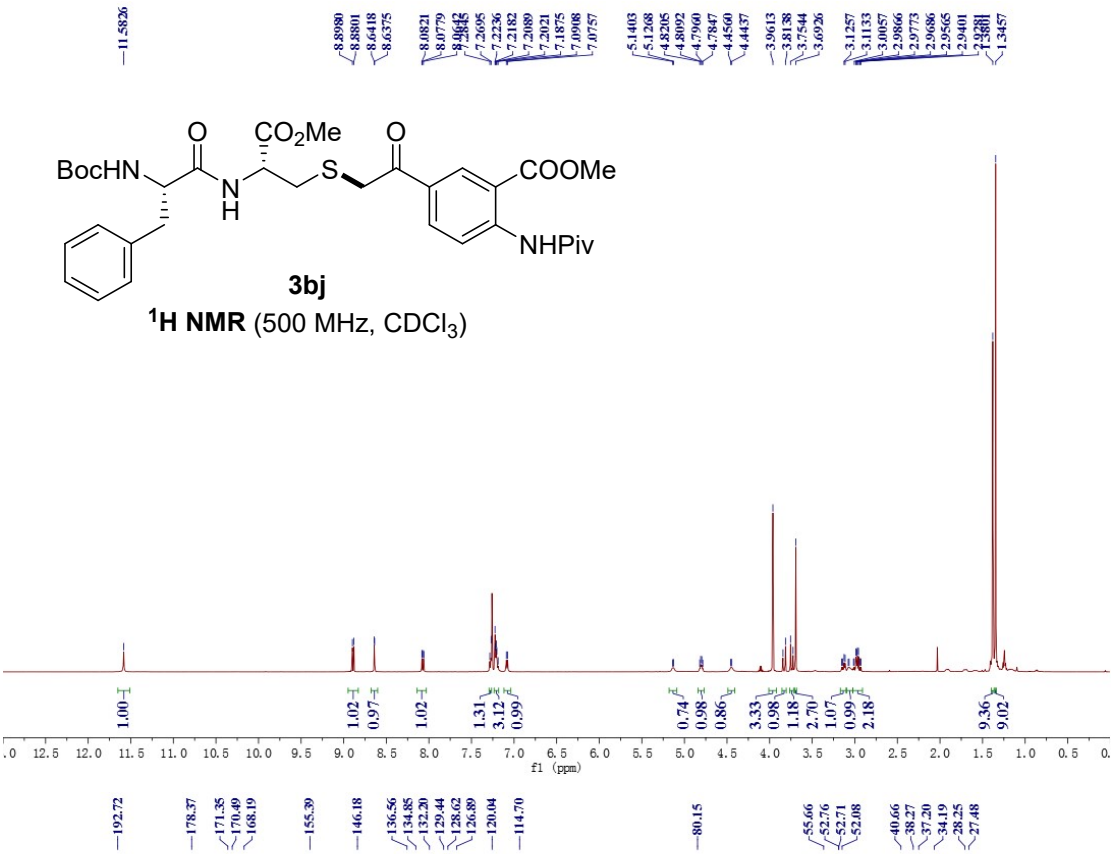
^{13}C NMR (100 MHz, $CDCl_3$)



^{1}H NMR (400 MHz, $CDCl_3$)

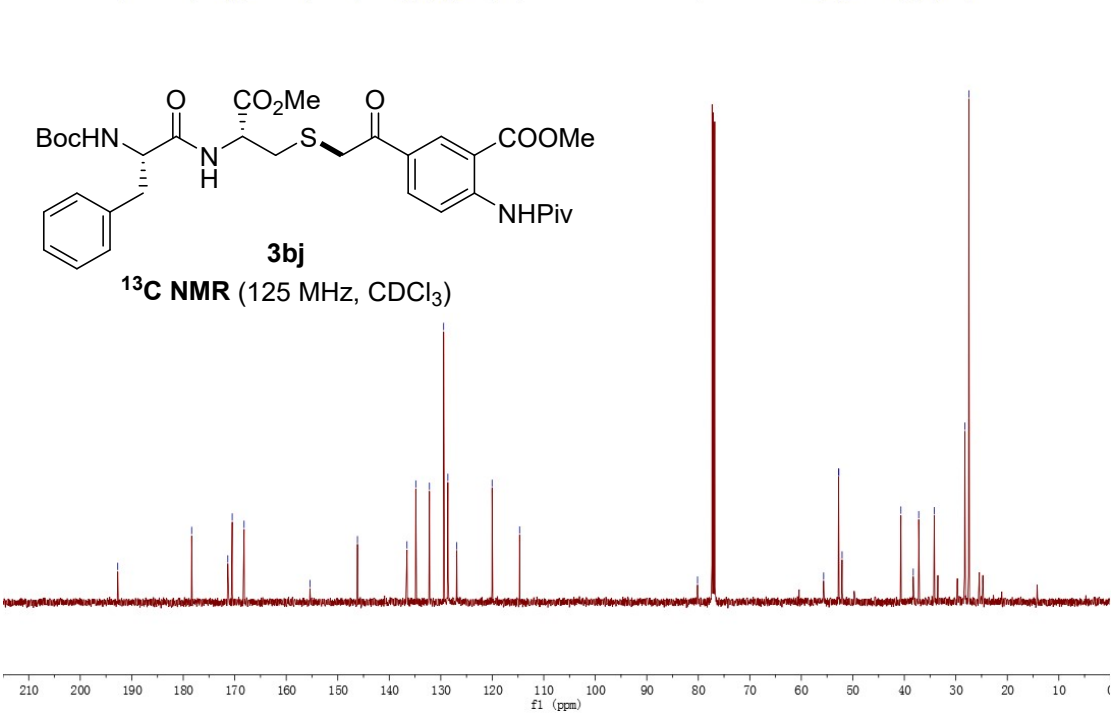


^{13}C NMR (100 MHz, $CDCl_3$)



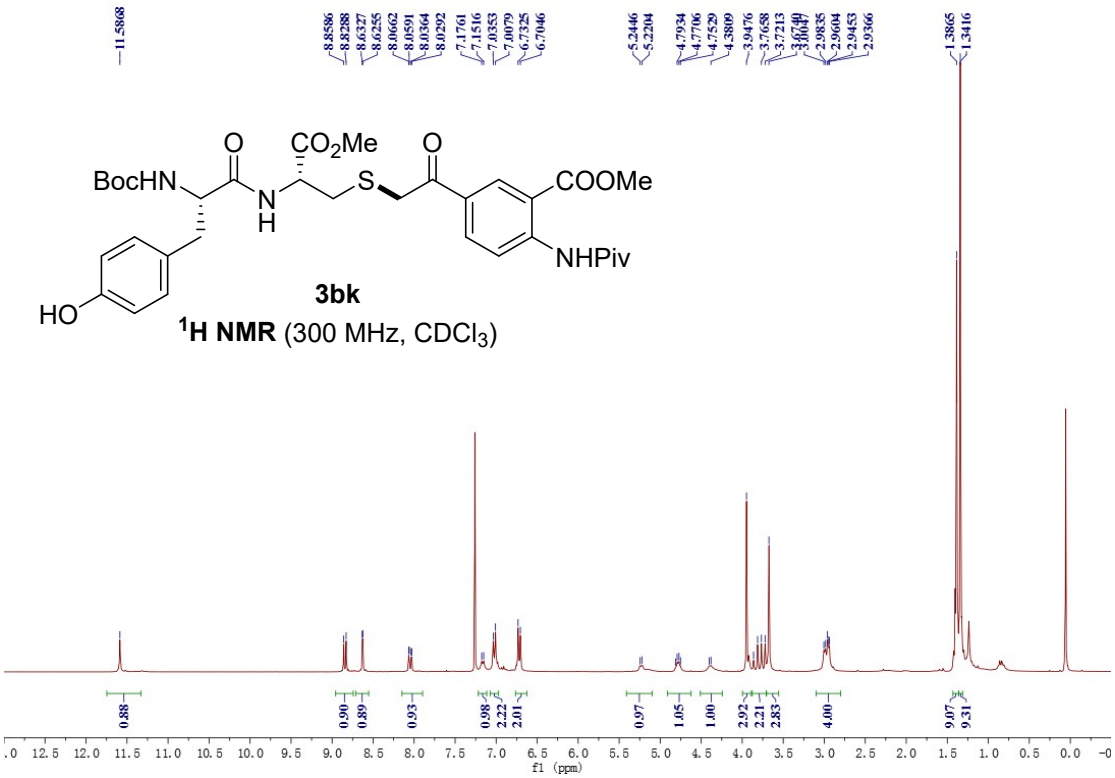
3bj

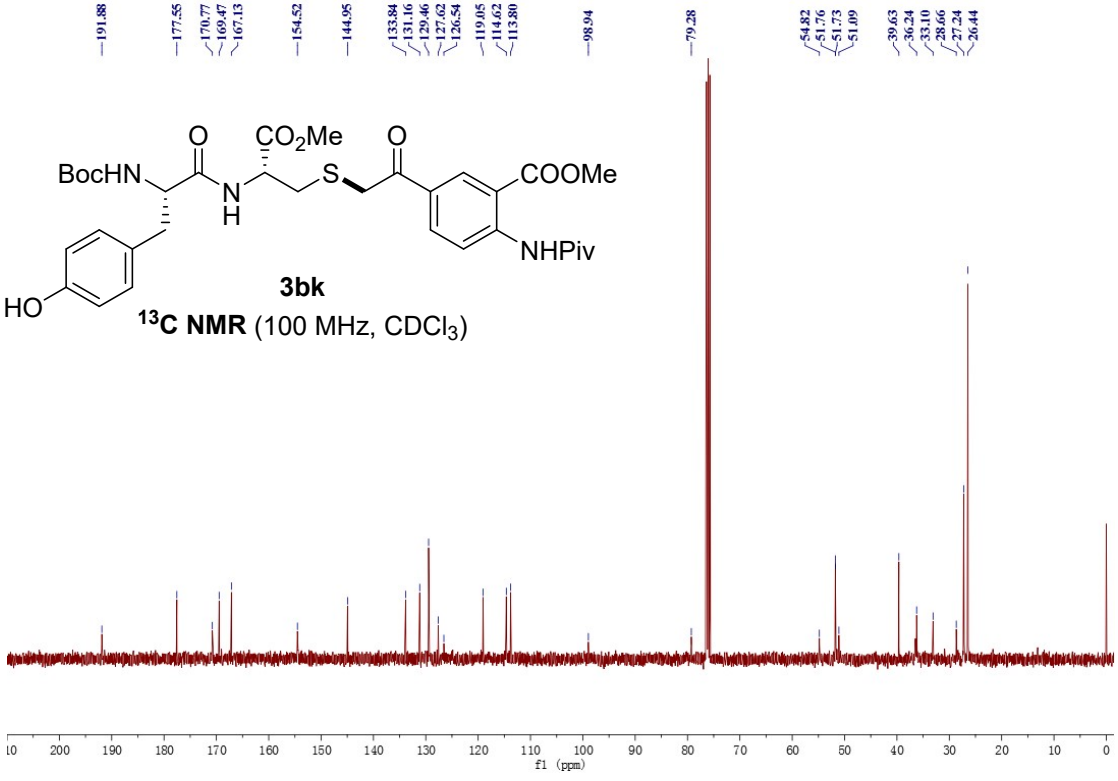
^{1}H NMR (500 MHz, $CDCl_3$)

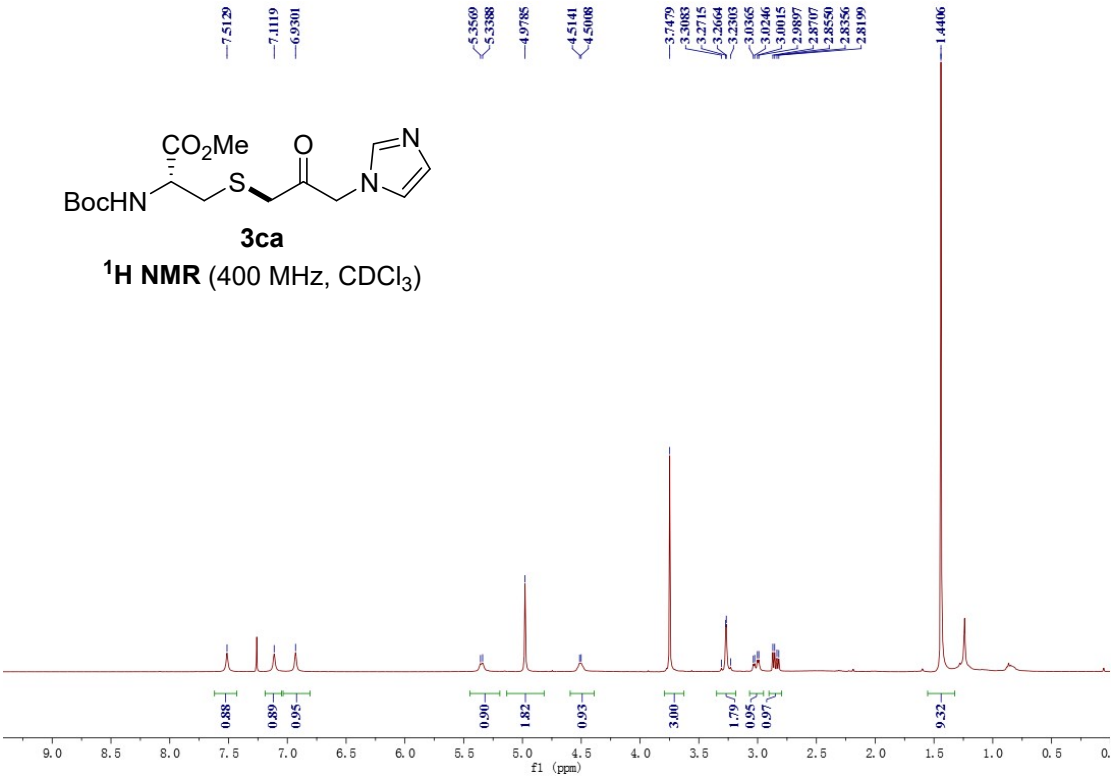


3bj

^{13}C NMR (125 MHz, $CDCl_3$)

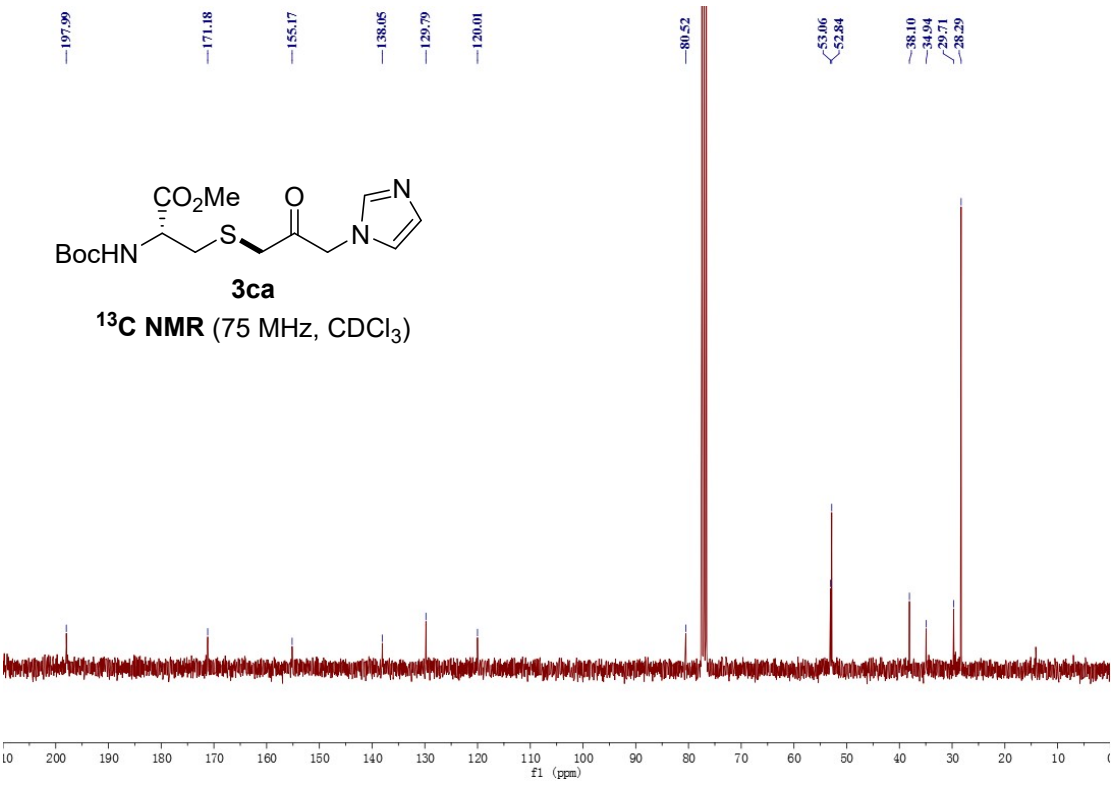

BocHN
O
CO2Me
O
N
H
S
O
COOMe
NHPiv
HO
3bk
1H NMR (300 MHz, CDCl3)
f1 (ppm)


BocHN
O
CO2Me
O
N
H
S
O
COOMe
NHPiv
HO
3bk
13C NMR (100 MHz, CDCl3)
f1 (ppm)



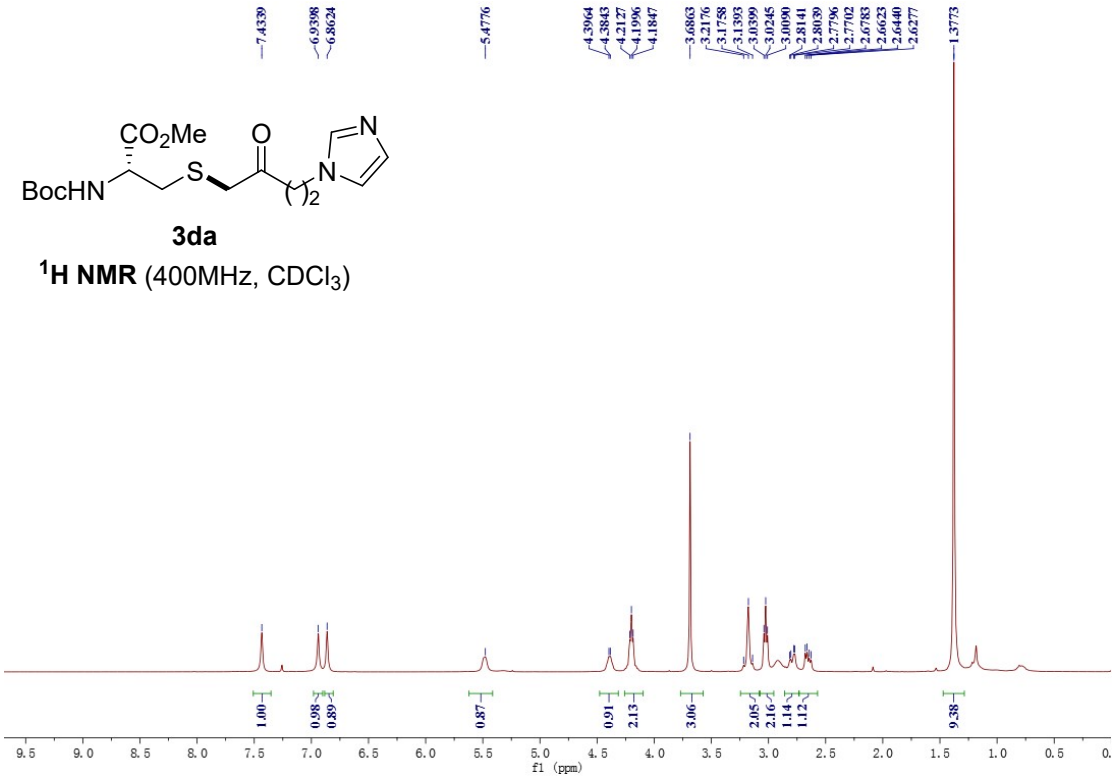
3ca

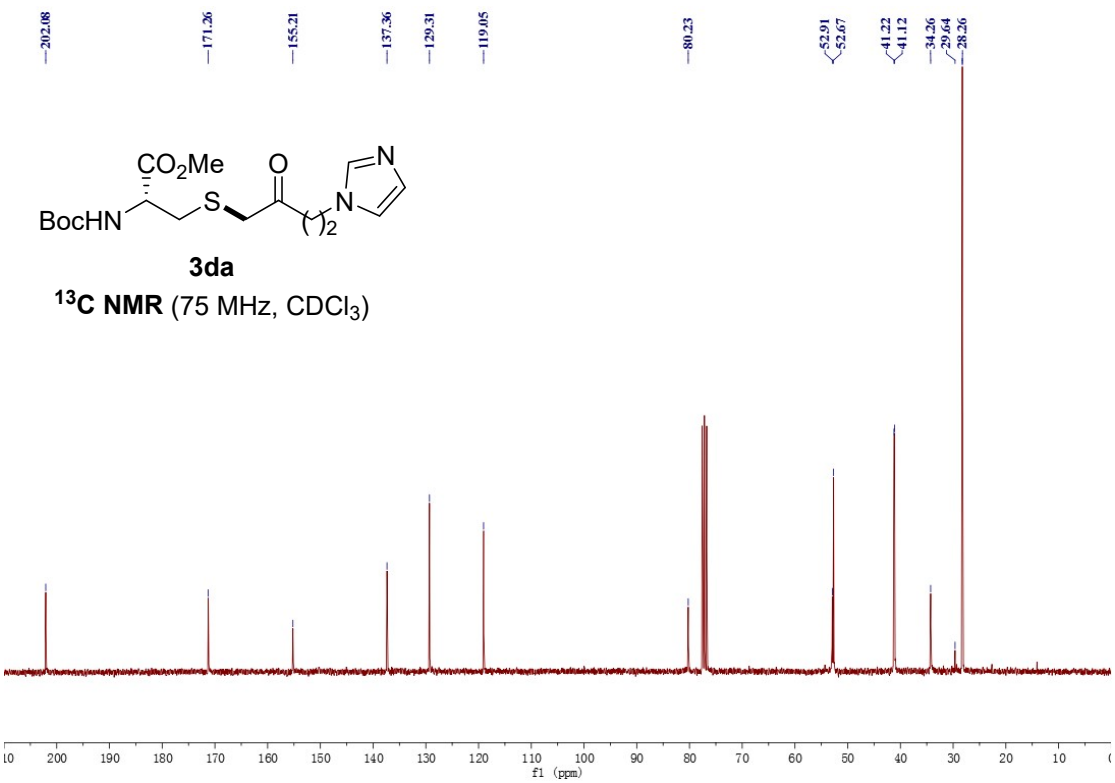
^{1}H NMR (400 MHz, $CDCl_3$)

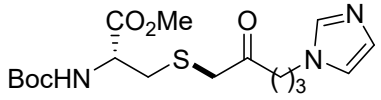


3ca

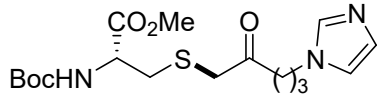
^{13}C NMR (75 MHz, $CDCl_3$)


7.4339
6.9398
6.8624
5.4776
4.3964
4.3843
4.2127
4.1996
4.1847
3.6863
3.2176
3.1758
3.1393
3.0399
3.0245
3.0090
2.8141
2.8039
2.7796
2.7702
2.6783
2.6623
2.6440
2.6277
1.3773
1.00
0.98
0.89
0.87
0.91
2.13
3.06
2.05
2.16
1.14
1.12
9.38
f1 (ppm)

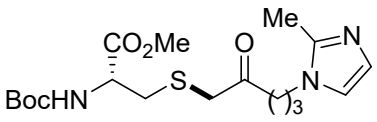

202.08
171.26
155.21
137.36
129.31
119.05
80.23
52.91
52.67
41.22
41.12
34.26
29.64
28.26
f1 (ppm)



^{1}H NMR (400 MHz, $CDCl_3$)

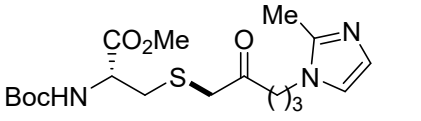


^{13}C NMR (100 MHz, $CDCl_3$)



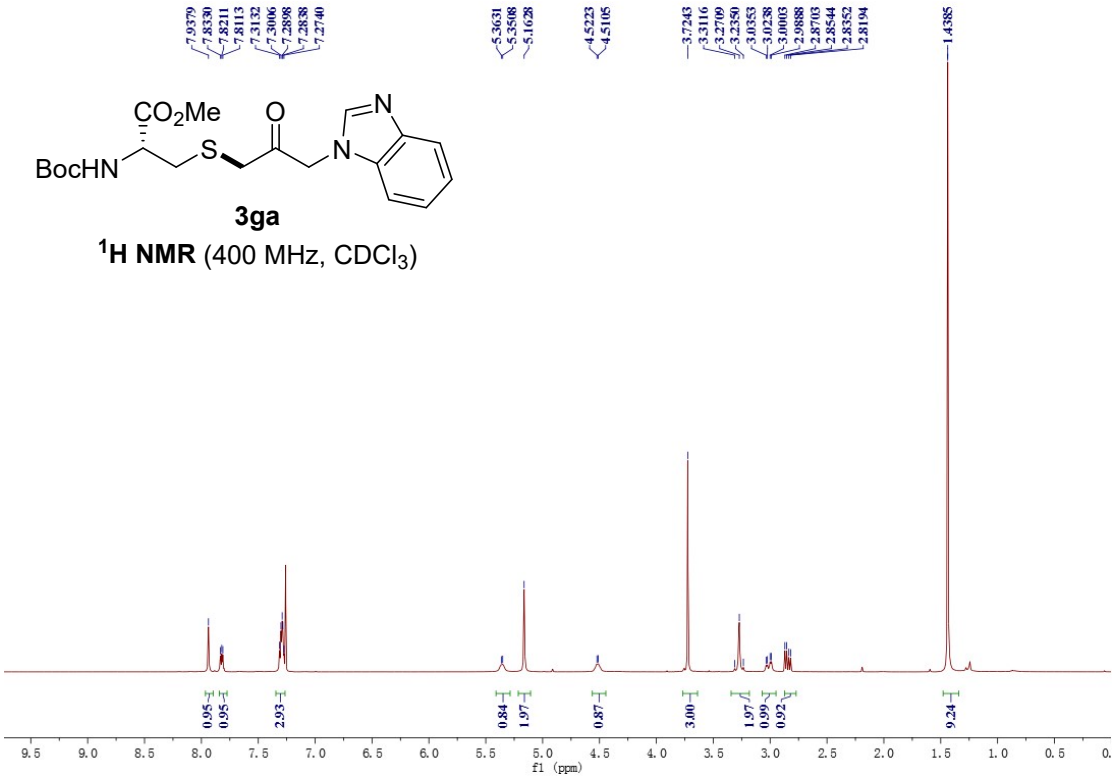
3fa

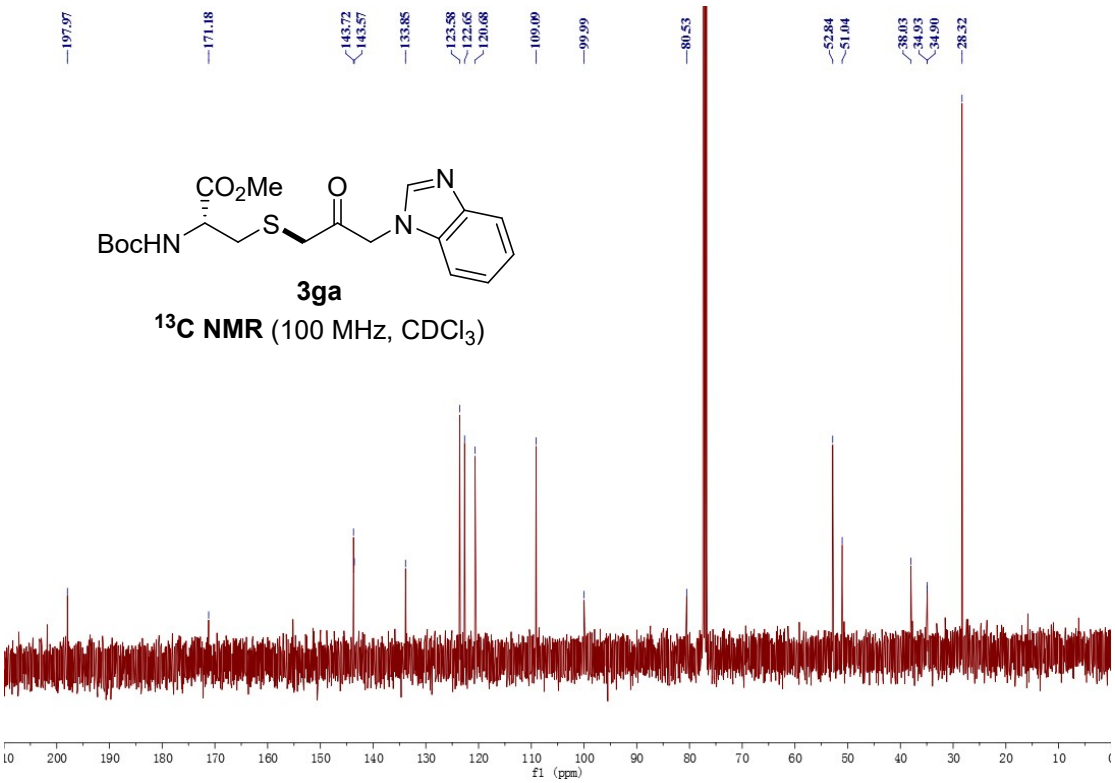
^{1}H NMR (400 MHz, $CDCl_3$)



3fa

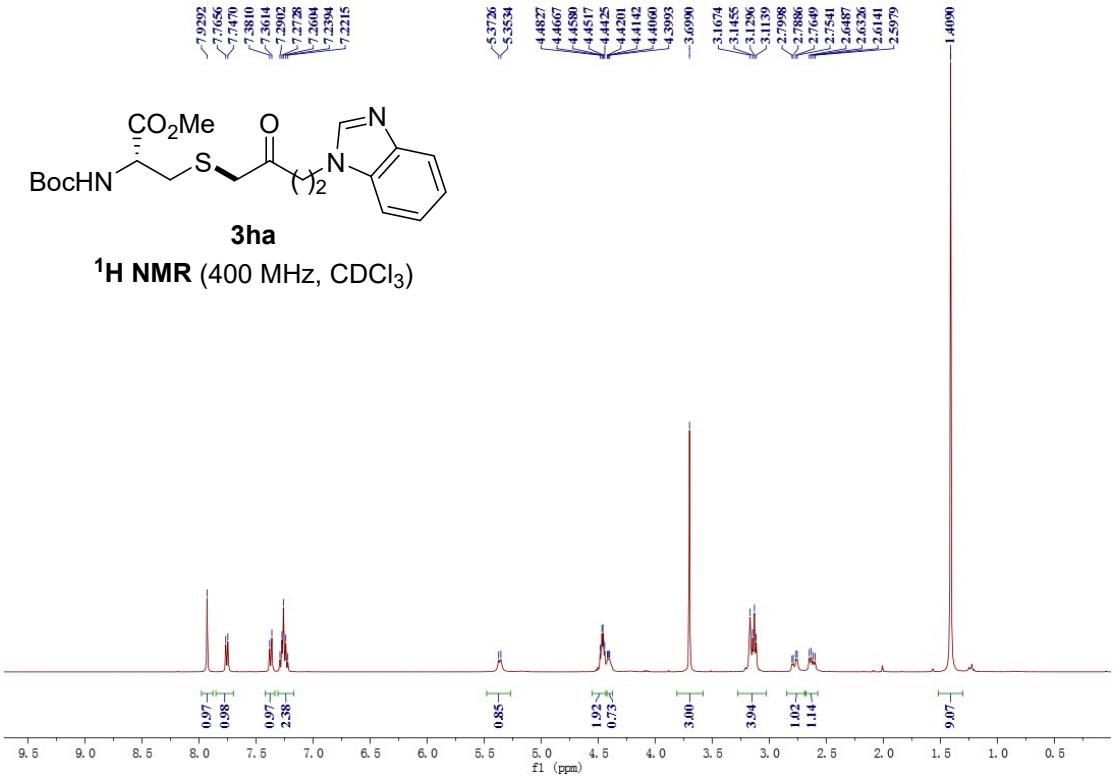
^{13}C NMR (100 MHz, $CDCl_3$)





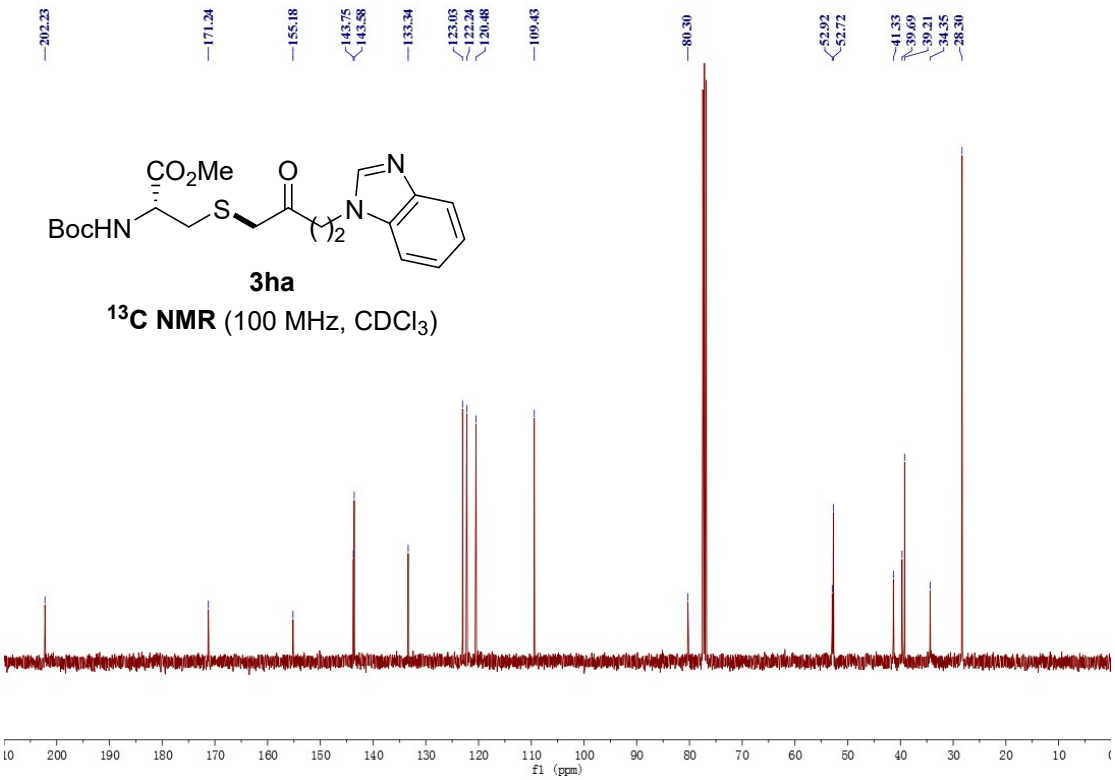
3ga

^{13}C NMR (100 MHz, $CDCl_3$)



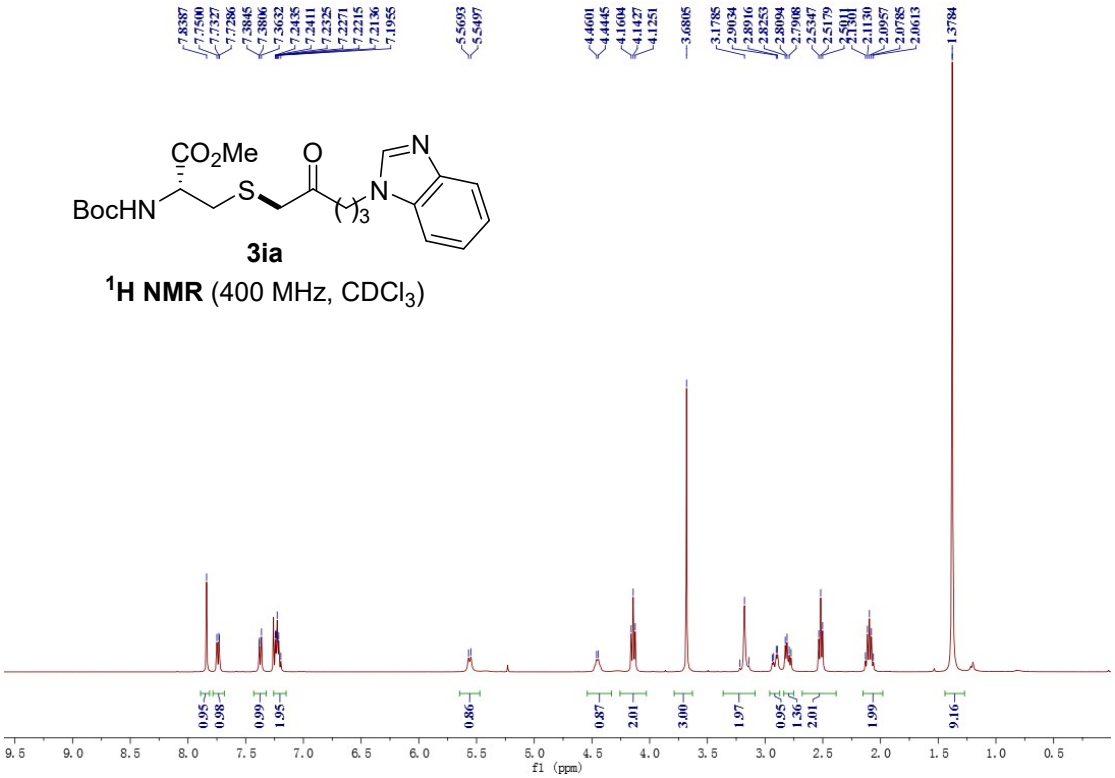
3ha

^{1}H NMR (400 MHz, $CDCl_3$)



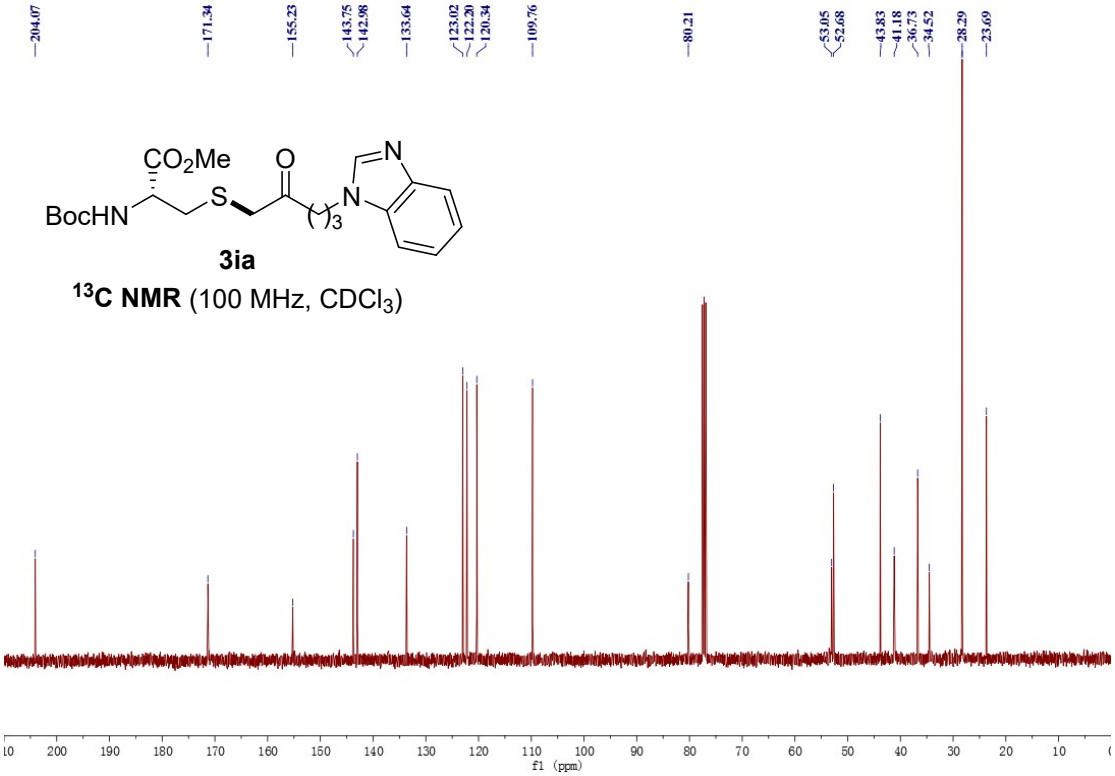
3ha

^{13}C NMR (100 MHz, $CDCl_3$)



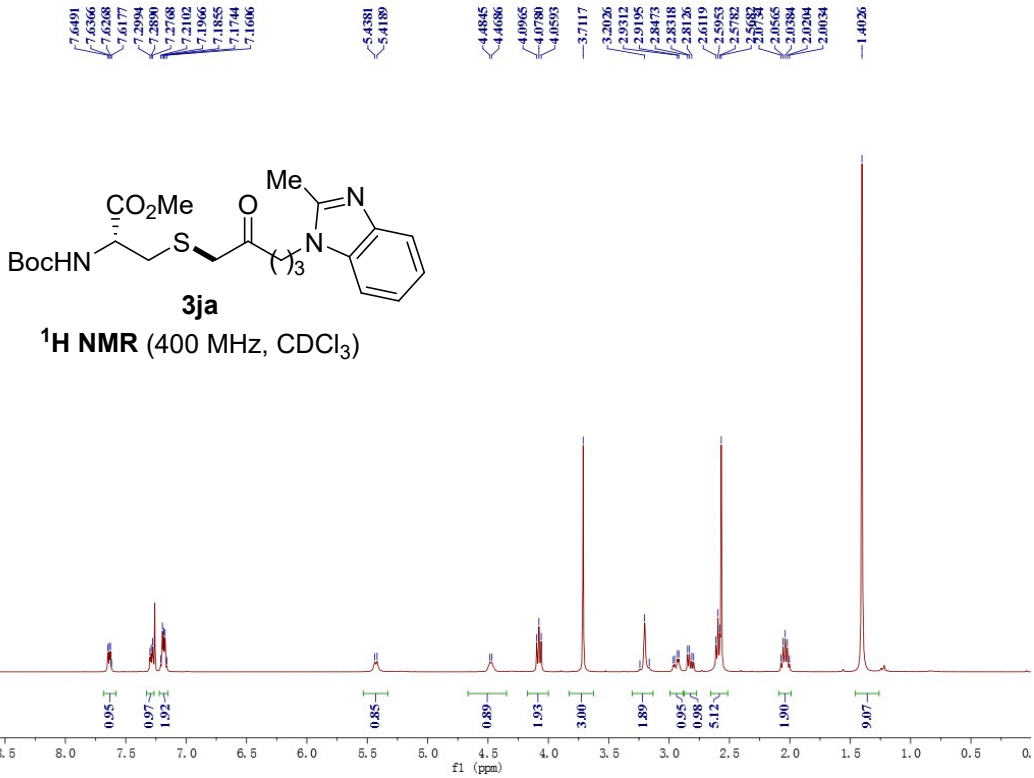
3ia

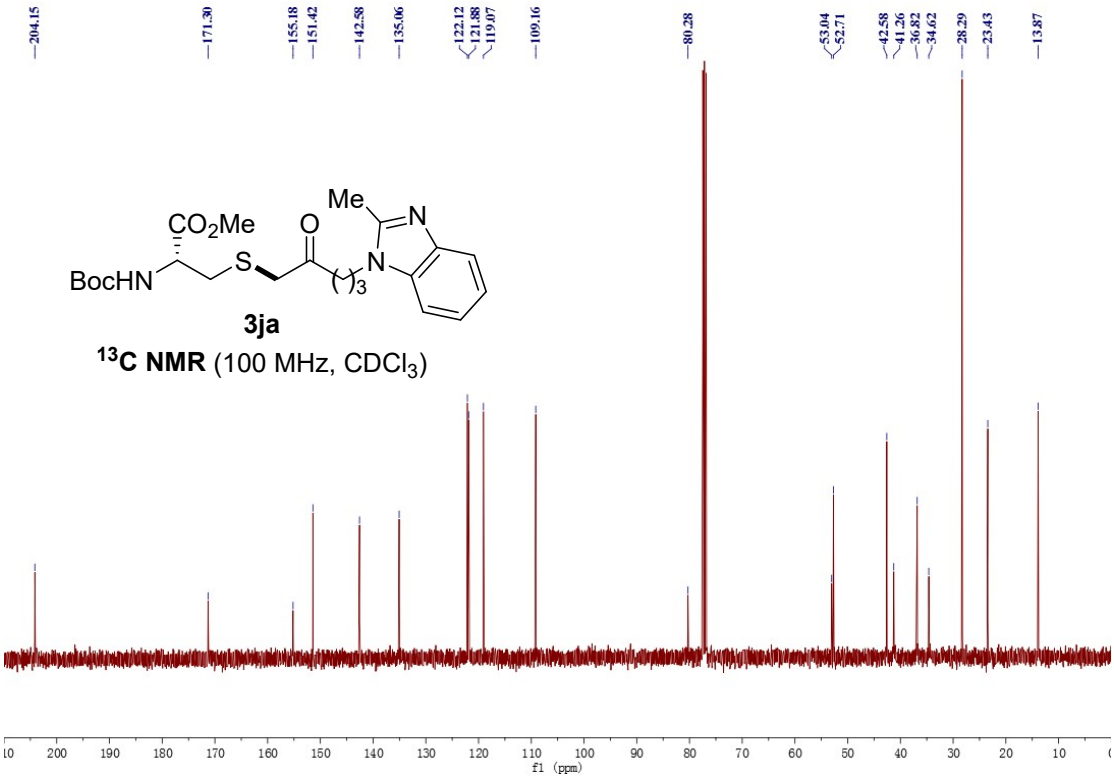
^{1}H NMR (400 MHz, $CDCl_3$)

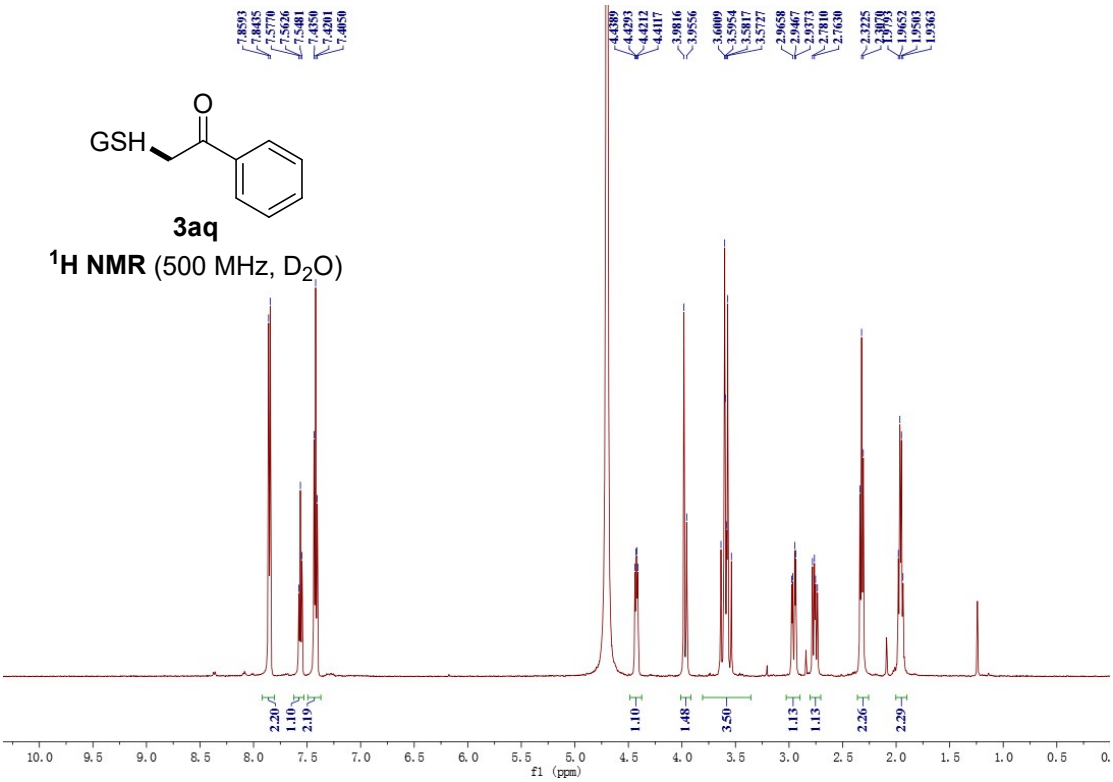


3ia

^{13}C NMR (100 MHz, $CDCl_3$)

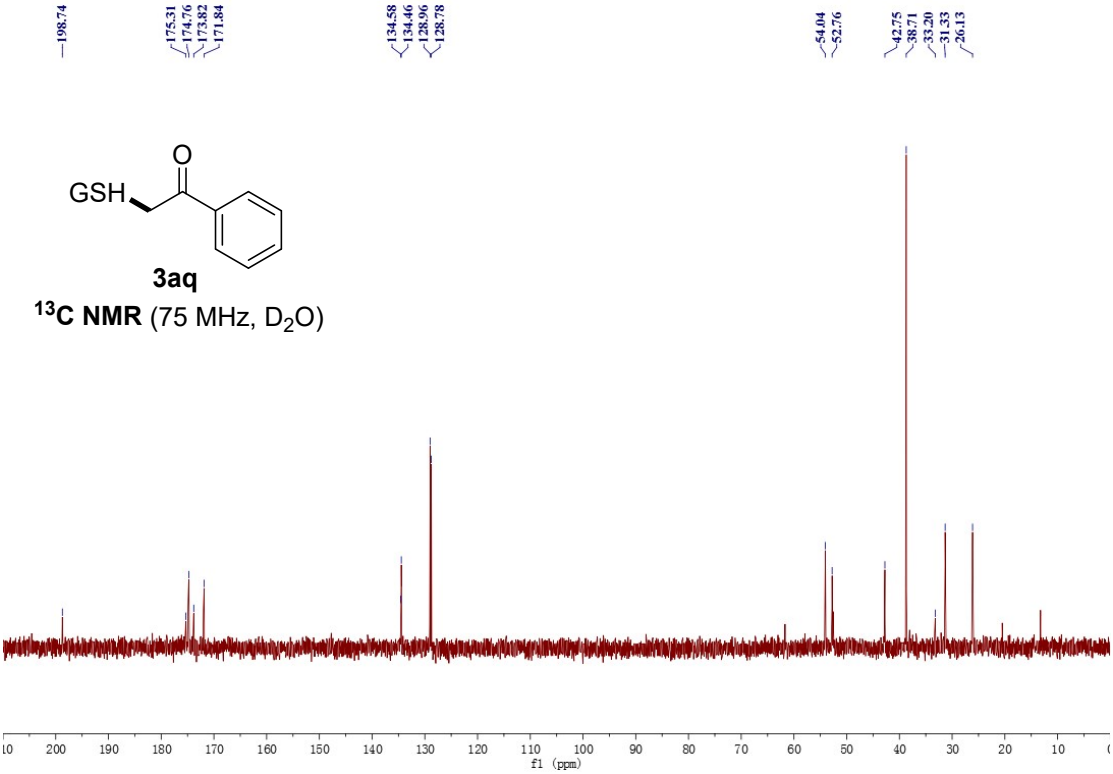

7.6491
7.6366
7.6268
7.6177
7.2994
7.2890
7.2768
7.2102
7.1966
7.1855
7.1744
7.1606
5.4381
5.4189
4.4845
4.4686
4.0965
4.0780
4.0593
3.7117
3.2026
2.9312
2.9195
2.8473
2.8318
2.8126
2.6119
2.5953
2.5782
2.0565
2.0384
2.0204
2.0034
1.4026
Me
N
CO2Me
O
BocHN
S
N
3
3ja
1H NMR (400 MHz, CDCl3)
0.95
0.97
1.92
0.85
0.89
1.93
3.00
1.89
0.95
0.98
5.12
1.90
9.07
f1 (ppm)


204.15
171.30
155.18
151.42
142.58
135.06
122.12
121.88
119.07
109.16
80.28
53.04
52.71
42.58
41.26
36.82
34.62
28.29
23.43
13.87
Me
N
CO2Me
O
BocHN
S
N
3
3ja
13C NMR (100 MHz, CDCl3)
f1 (ppm)



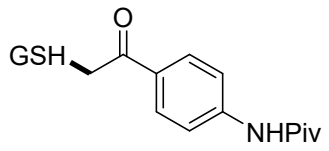
3aq

^{1}H NMR (500 MHz, D_2O)



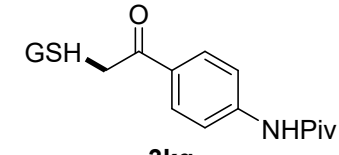
3aq

^{13}C NMR (75 MHz, D_2O)



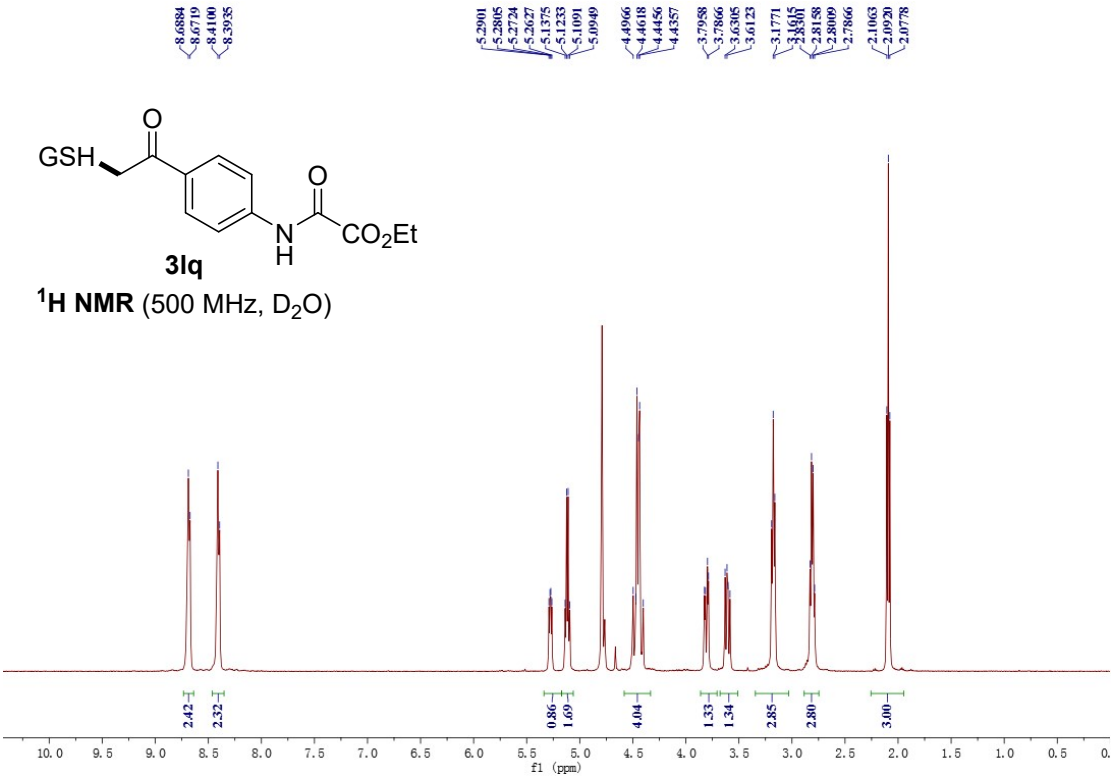
3kq

^{1}H NMR (400 MHz, D_2O)



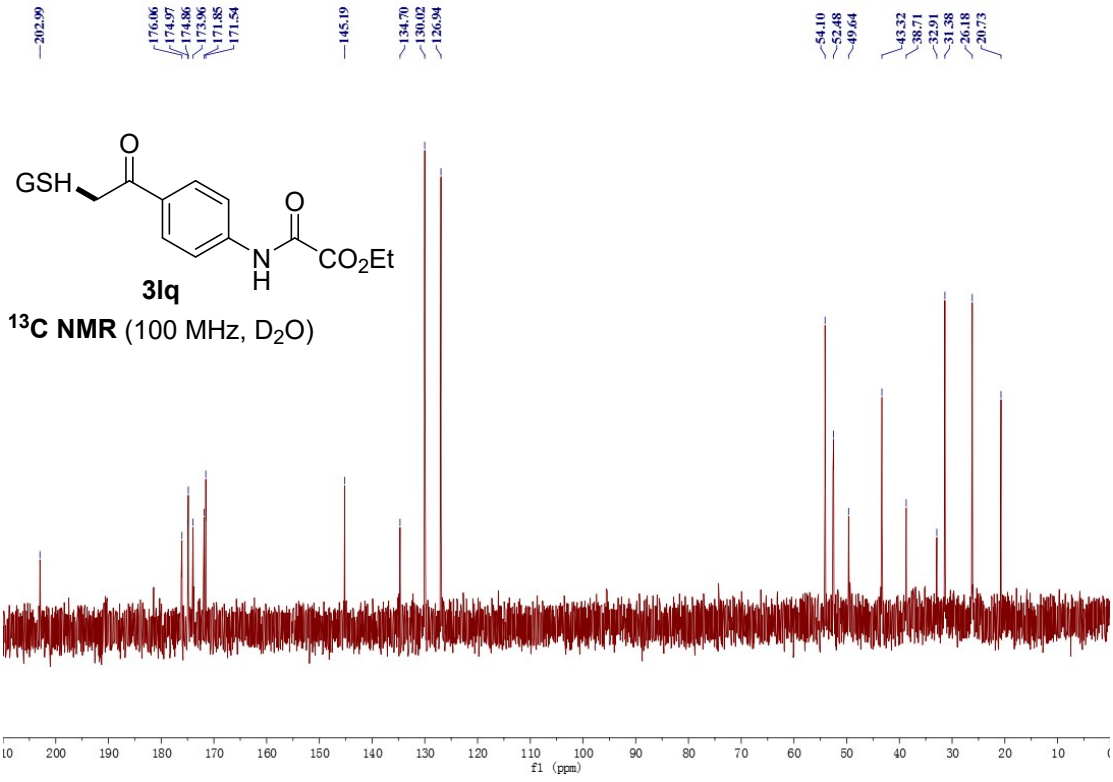
3kq

^{13}C NMR (100 MHz, D_2O)



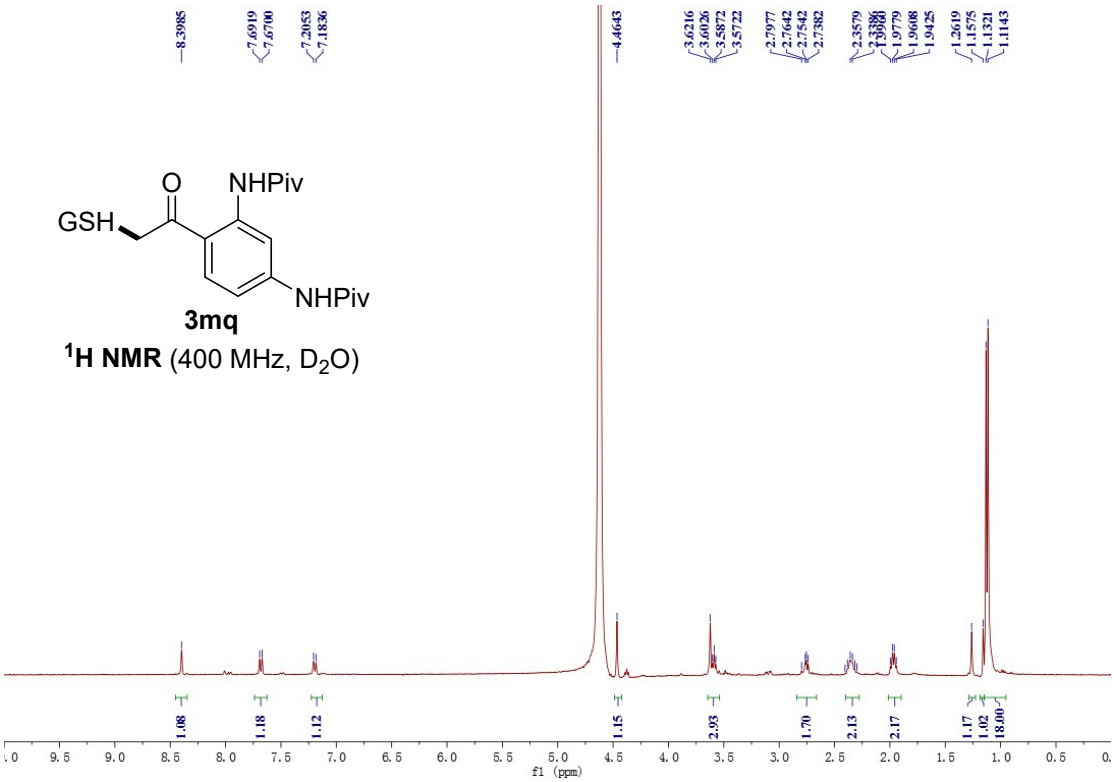
3Iq

^{1}H NMR (500 MHz, D_2O)



3Iq

^{13}C NMR (100 MHz, D_2O)



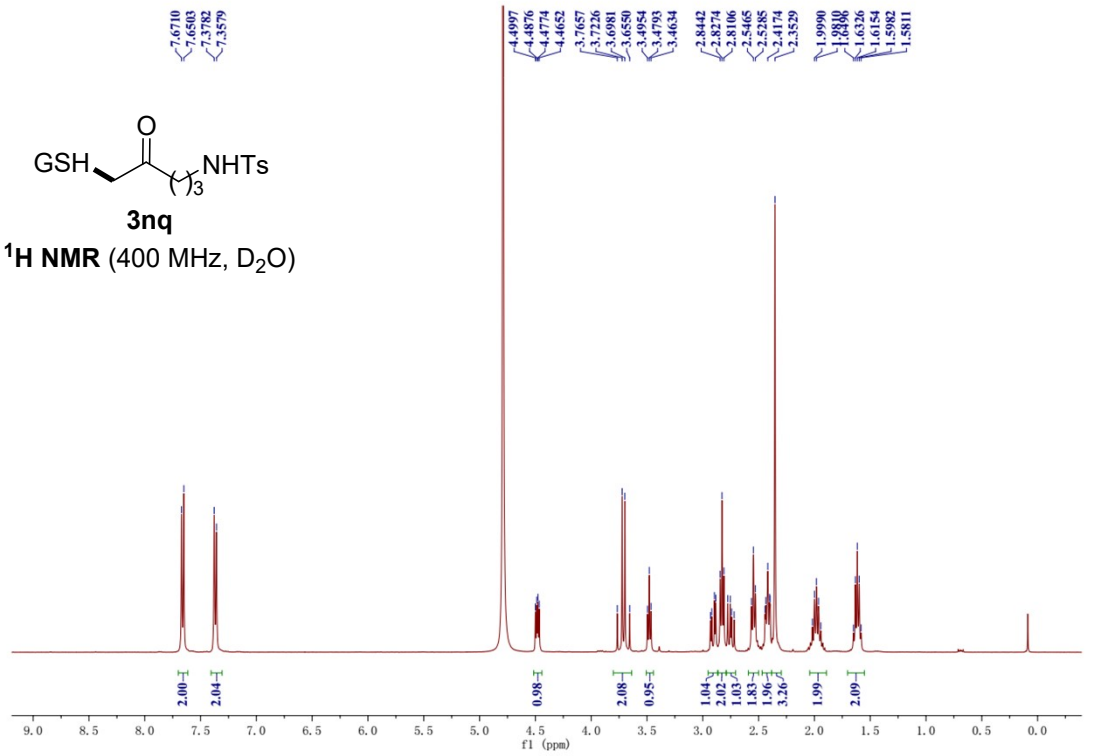
O NHPiv

GSH

NHPiv

3mq

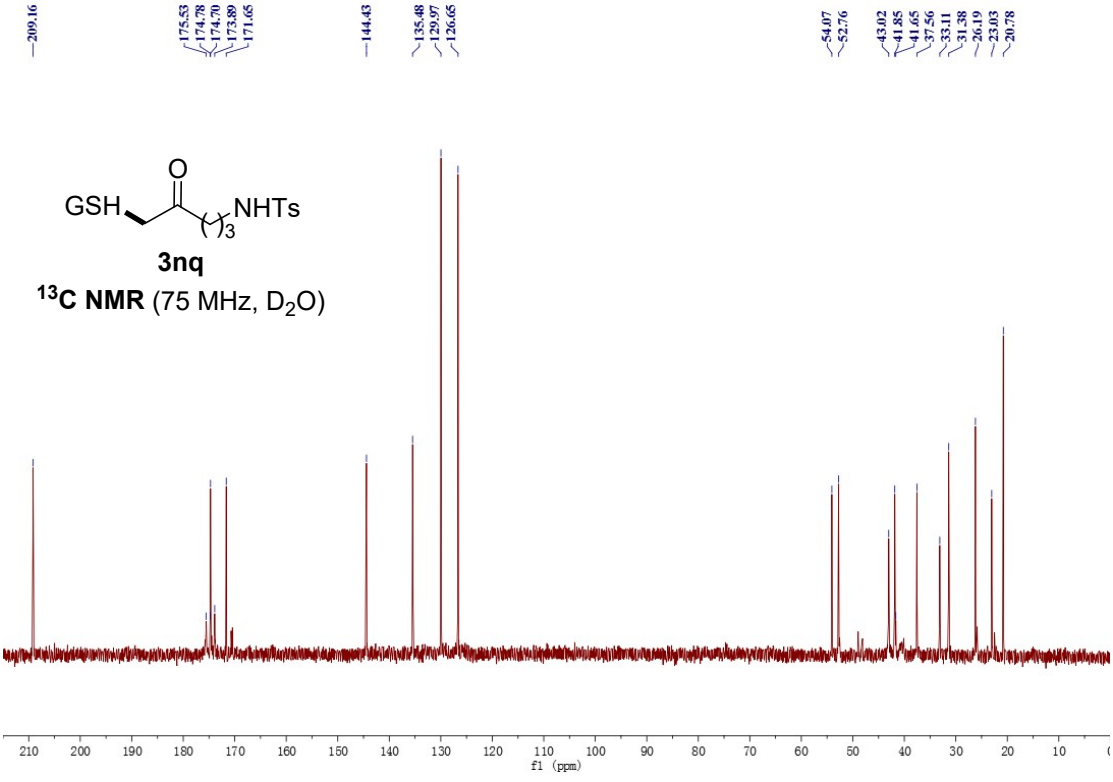
^{1}H NMR (400 MHz, D_2O)



GSH NHTs

3nq

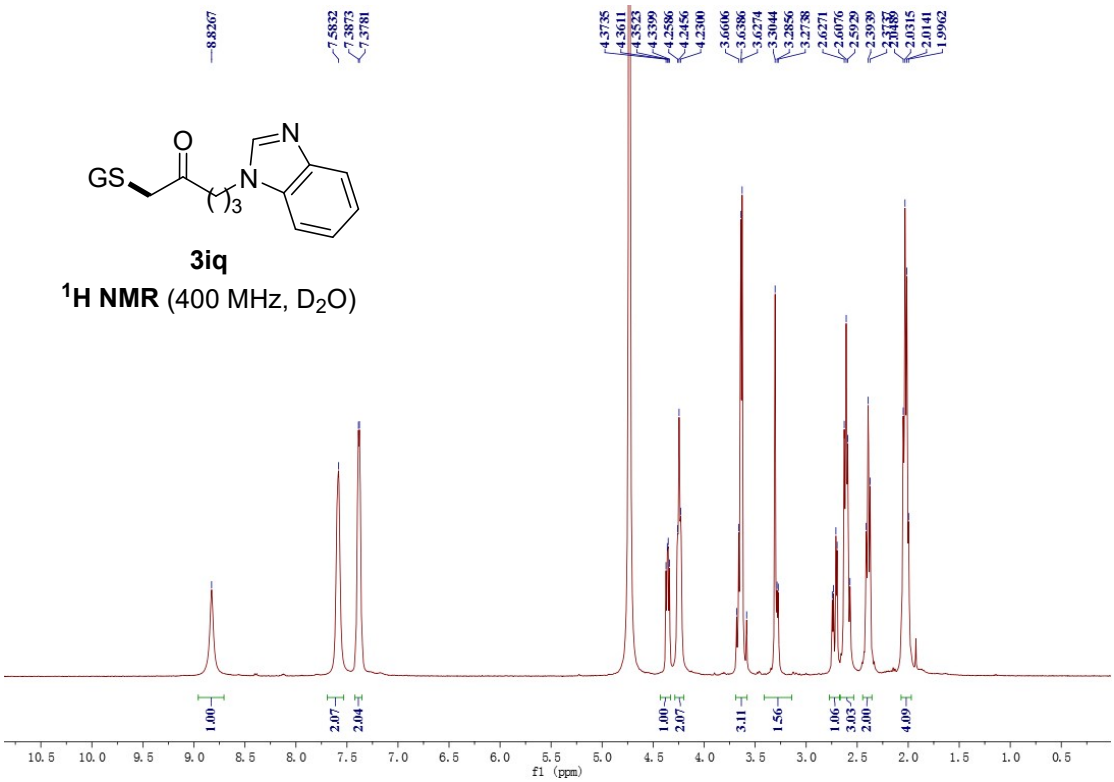
^{1}H NMR (400 MHz, D_2O)

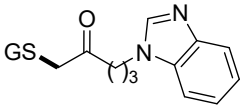


GSH NHTs

3nq

^{13}C NMR (75 MHz, D_2O)



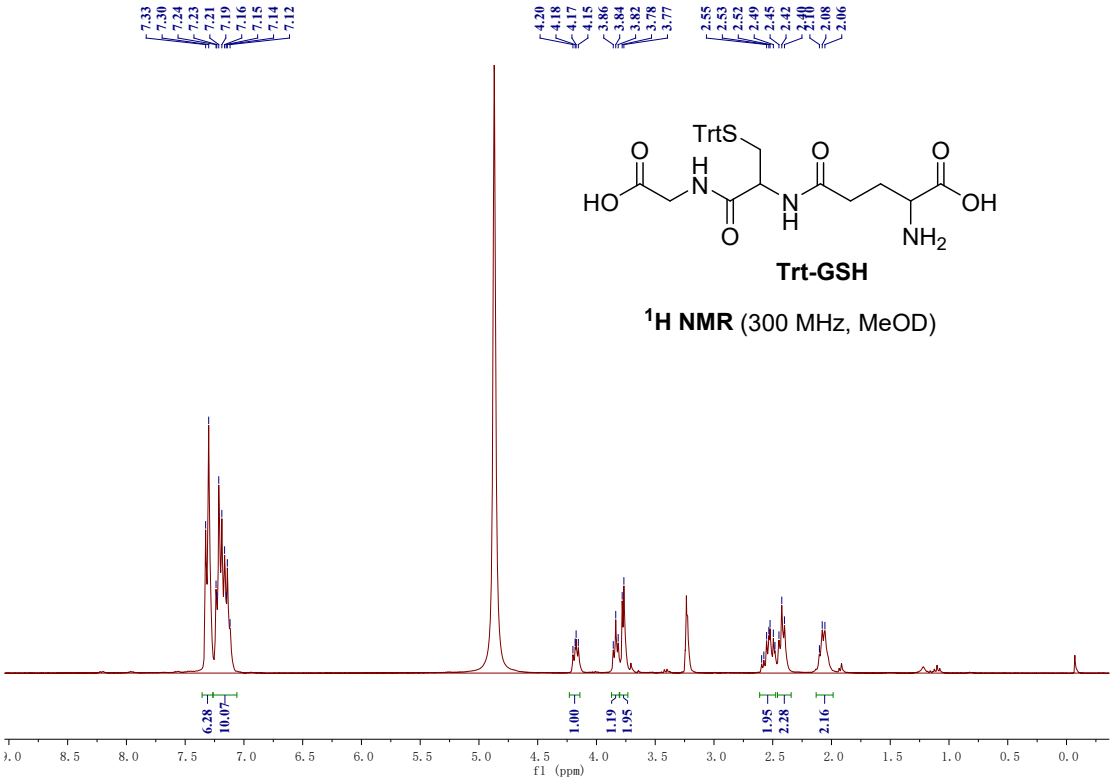


3iq

^{1}H NMR (400 MHz, D_2O)

3iq

^{13}C NMR (100 MHz, D_2O)



Trt-GSH

^{1}H NMR (300 MHz, MeOD)